Assessment of Health Impacts of Particulate Matter from Indoor Air Sources Phase I: Development of In Vitro Methodology

Final Report

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ABSTRACT

The overall objective of this study was to develop approaches to assess the toxicities of several major indoor PM source samples by using human *in vitro* cell models with a focus on inflammatory and oxidative stress responses. In the pilot study, the capacity of extracts from incense PM to stimulate inflammatory marker production in four *in vitro* human cell models was evaluated. The U937 macrophage cell line was the most sensitive of the test models followed by the NCI-H441 bronchiolar Clara cell line.

PM toxicity from cooking activities, candle burning, wood burning, and incense burning was assessed in both cell models. All indoor PM sample source types had some positive response in either or both of the human cell lines with incense producing the largest responses. In further analytical studies, it was determined that incense PM contained high levels of PAHs while woodsmoke had lower levels. Incense also was found to contain many other compounds such as vanillin which may contribute to its high toxicity. Woodsmoke yielded hydrocarbons containing a series of siloxanes. Cooking PM sample from the stir-frying contained mainly hydrocarbons related to the oil used. The candle samples consisted of numerous hydrocarbons such as alkanes and alkenes.

The information from this study will help ARB in the overall assessment of health risk from these indoor sources by providing toxicity data using human cell systems, and provide some initial information for future health effects studies.

EXECUTIVE SUMMARY

Introduction and Background

Among the indoor pollutants reviewed by ARB, PM is considered to be especially hazardous to those who are chronically exposed. There are very few studies of the health impacts of indoor PM and a substantial portion of indoor PM is likely derived from indoor sources (Zhang and Smith 2003). The health risks associated with indoor PM could potentially be even greater than outdoor PM for those individuals such as infants and the elderly who must spend most of their time in indoor environments. However, toxicity profiles from the various indoor PM sources have not been studied systematically. The chemical and toxicological properties of indoor-generated PM could be similar to or very different from those of outdoor PM, since in most cases indoor sources are located near occupants, and these sources could be unique for indoor use. Further, indoor PM is less subjected to atmospheric chemical transformation or degradation by UV and extreme temperatures. One approach to study the health effects of indoor PM is to initiate extensive animal toxicity test programs. However, such studies require substantial resources, time, analyses, and generally large quantities of sample. An alternative approach is to use established *in vitro* cell culture methods. With this approach, it is important to design a target cell-based study (especially if based on human cells) to detect markers of toxicities of indoor PM, including those indicating potential deleterious effects on respiratory and cardiovascular health.

Methods

In the pilot study, cells were exposed to extracts of the PM from urban dust particles and incense. Expressions of inflammatory and oxidative stress markers were measured. Initially, four *in vitro* human cell models consisting of the two main target cell types for PM, human macrophage cells and lung cells, were tested. U937 macrophages were found to be the most sensitive of the cell types tested. NCI-H441, a bronchiolar Clara cell line, was found to be the most sensitive lung cell type when compared to the human alveolar lung cell line, A549, and HPL1 cells, and a normal lung epithelial cell line. The macrophage and the Clara cell lines were then used for the indoor PM samples throughout this study.

For the main study, methods were devised to collect PM samples from the following indoor sources: 1) cooking activities 2) candle burning 3) wood burning, and 4) incense burning. The most potent PM samples identified by the biological tests were subjected to further examination using bioassay-directed fractionation to chemically characterize the most toxic components. Gas chromatography/mass spectrometry (GC/MS) analysis was used for chemical characterization of the compounds present in the indoor source PM. The following flow diagram is provided to summarize the procedures used in the main study.

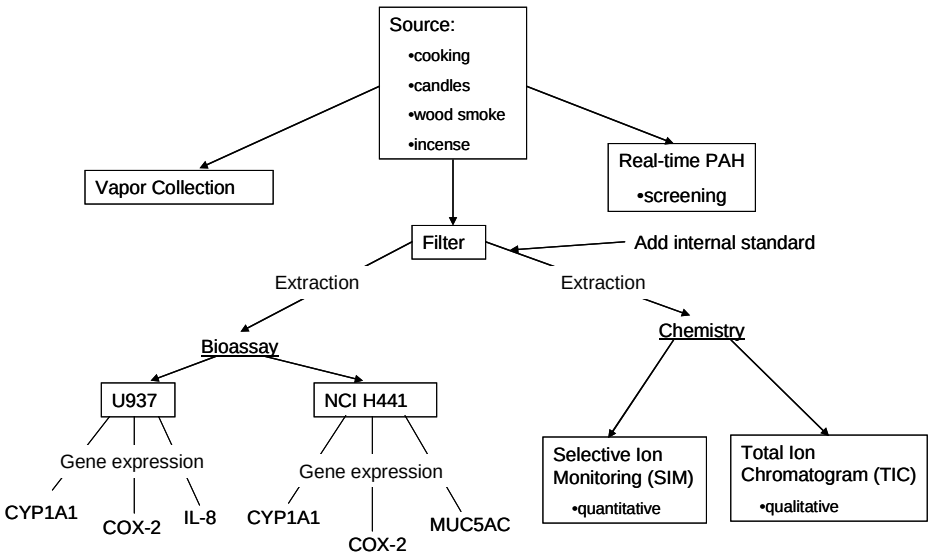


Figure 1. Flow diagram summarizing procedures for the Main Study.

Results

The biologic markers tested included the xenobiotic metabolizing enzyme cytochrome P4501A1 (CYP1A1), the inflammatory enzyme cyclooxygenase 2 (COX-2), the chemokine interleukin 8 (IL-8), and heme oxygenase-1 (HO-1). Of the PM source samples collected, the combustion products of incense had the highest response in the cells, with PM samples derived from candles, woodsmoke, and cooking having lower, but measurable responses. The incense samples were the most potent indoor PM found in the biological tests, and so were subjected to a more in-depth investigation to chemically characterize the components contributing to their biological activity. The PM extracts from the incense were fractionated and results showed the most active fraction was the most polar fraction.

Initial real-time monitoring of PAHs also indicated that wood- and incense-burning emit high levels of particle-bound PAHs. Quantitative chemical analysis of PAHs was performed on these and the other indoor PM source samples using GC/MS. For woodsmoke, the vapor-phase sample contained some detectable PAHs. In the incense PM, several PAHs were detected at high concentrations and were comparable to or possibly even higher than in the standard reference diesel particles (NIST SRM 2975).

Qualitative chemical characteristics of compounds were also evaluated with GC/MS for the indoor PM source samples. Major compounds present were different in the different PM source samples measured. For cooking, the compounds in the stir-fry PM sample reflected components found in cooking oil. The candle PM contained hydrocarbons such as alkanes and alkenes. The woodsmoke had hydrocarbons containing a series of siloxanes. For the incense, GC/MS analyses indicated that some of the compounds present in its most polar

fraction were carbonyls, substituted nitrophenols, and substituted bromobenzenes.

Conclusions

Based on the results of this study, a number of conclusions were drawn.

First, two human cell systems, macrophage cells and lung cells (Clara type), were very sensitive to the expression of markers for inflammation and oxidative reactions.

Second, several indoor PM source samples were acquired, prepared, and integrated for analysis in the human cell bioassay. All indoor samples had some activity in at least one of the cell types and at least one marker. The most potent indoor source PM was derived from incense, followed by woodsmoke, candles, and cooking PM.

Third, chemical fractionation based on polarity of the complex mixture of incense PM extract was conducted and each fraction tested in the human cell systems. Although all of the fractions induced detectable levels of markers of inflammation, the most potent fraction was the most polar (methanol) fraction.

Fourth, the levels of PAHs in the incense first detected by the real-time PAH instrumentation were confirmed chemically by GC/MS analyses of the extracts. The PAH levels in the incense samples were higher than other indoor PM source samples, but PM levels of the other samples were limited in levels compared to the incense samples overall. For the incense, PAHs are present in chemical fractions that precede the most polar (most active) fraction.

Finally, the integrated study of indoor source PM samples with human cell assay systems can provide a unique survey and evaluation regarding potential inflammatory response and oxidative stress reactions which are relevant for PM-related health effects.

1. INTRODUCTION

Background

Exposure to airborne particulate matter (PM) is a health concern for the people of California, since many serious health effects associated with exposure to air pollution are thought to be related to PM exposure. Moreover, epidemiological evidence indicates that fine particulate matter (PM2.5) is associated not only with respiratory diseases, but also cardiovascular diseases and possibly cancer (Pope et al., 2004; Brook et al., 2003; Ostro et al., 1999; Simkhovich et al., 2008; Valavanidis et al., 2008). Indoor air pollution is of concern since people spend most of their time indoors. For example, Californians are reported to spend approximately 80 to 90 percent of their time indoors (Jenkins et al., 1992). PM is one of many potentially toxic indoor pollutants, and the health risks associated with PM exposures could be even greater in the indoor environment than outdoors for individuals, such as infants and the elderly, who must spend most of their time indoors. A report to the California Legislature prepared by the Air Resources Board recommended that indoor pollutants and their sources should be ranked in the high-risk category (CARB, 2005). However, toxicity profiles from the various indoor PM sources have not been studied systematically.

It has been reported that a substantial portion of indoor PM is likely derived from indoor sources (Zhang and Smith, 2003). Indoor pollutants include those produced through combustion, chemical aerosols, and dust containing biological materials as well as non-biological matter. Among them, some of the major contributors to indoor PM are combustion sources such as smoking, cooking, burning of wood, and candles ((Zhang and Wallace et al., 2003; Ozkaynak et al., 1996; Brauer et al., 2000; Abt et al., 2000a, 2000b; Fortmann et al., 2001). Also, incense burning has been reported to emit fine PM in large quantities compared to other indoor sources (Jetter et al., 2002). Indoor-source PM combustion products may not be significantly degraded or otherwise altered by ultraviolet light. Exposure from PM sources indoors may typically be different from outdoors, since people are more likely to be situated in closer proximity to indoor sources.

These characteristics led us to focus on “near source” indoor air samples since, unlike outdoor situations, indoor-generated pollutants to which people are exposed may not be chemically transformed as seen in the outdoor atmosphere. Sampling methods were developed to acquire near source indoor PM samples, which was realistic in terms of human exposure yet occurring under relatively well-controlled conditions. As major combustion sources for indoor PM, cooking, candle, woodsmoke, and incense burning were selected for investigation in this study. These near-source PM samples were extracted in a solvent, and then were biologically tested for their toxicities. Initial chemical characterization of the PM was also conducted.

Molecular Biomarkers

We used *in vitro* cell culture methods to provide some initial screening of indoor source PM toxicity. This approach was considered more suitable than animal toxicity test procedures for due to much lower investment in resources, time, analyses, and quantities of PM material required. It was therefore important to design a target cell-based study to detect biomarkers of toxicities of indoor PM, including those indicating potentially deleterious effects of PM on both respiratory and cardiovascular health.

In studies completed by us (Vogel *et al*., 2007) and others, PM and PM-related components have been shown to up-regulate (genetically initiate) a number of inflammatory and oxidative stress related biomarkers highlighted in bold in Figure 1-1 (explanations of the abbreviations are provided in the following text).

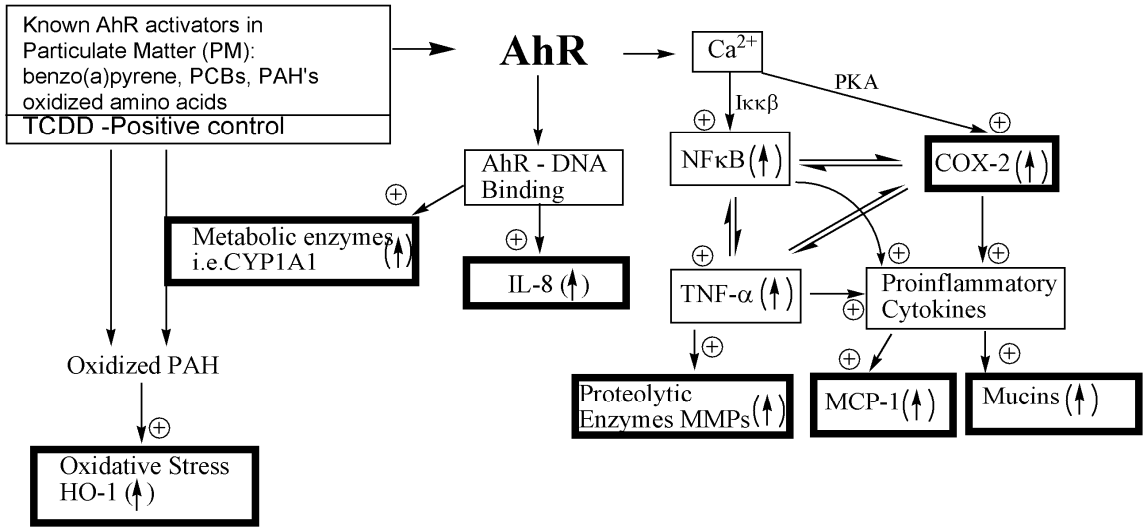


Figure 1-1. Model relationship for molecular biomarkers of inflammation and oxidative stress.

In this model, PM-mediated cellular toxicity is due to the ability of a number of PM-related components such as **polycyclic aromatic hydrocarbons (PAHs)** to bind to the **aryl hydrocarbon receptor (AhR).** At this point the "activated" AhR has been shown to increase the protein concentration of a number of metabolic enzymes such as **cytochrome P450 1A1 (CYP1A1)** through increased transcription via direct DNA binding. Our laboratory has also previously shown that **interleukin-8 (IL-8),** an inflammatory cytokine is regulated by a similar mechanism (Vogel et al., 2009).

Our lab has also recently shown that AhR activation can lead to molecular events even without direct transcriptional regulation (Sciullo *et al*, 2009). This pathway involves a large influx of calcium and the up-regulation of a number of pro-inflammatory prostaglandins , most notably **cycloxygenase-2 (COX-2)**. In the lung, up-regulation of COX-2 as well as pro-inflammatory cytokines has been shown to increase the production of a number of additional markers such as **mucin (MUC)**, a thick secretory protein involved in small airway disease, **monocyte chemoattractant protein -1 (MCP-1)**, which is responsible for monocyte (white blood cell) infiltration during injury, and **matrix metalloproteinases (MMPs)**, which are involved in lung remodeling and cellular differentiation.

In addition to inflammatory biomarkers, PM has been shown to increase the production of oxidative stress biomarkers such as **heme oxygenase-1 (HO-1).** This can occur by a PAH-mediated process (Li 2002) as well as via organic chemicals which occur in the PM vapor phase (Eiguren-Fernandez, 2010).

Cell strain selection

From previous work, we found that human **U937 macrophage cells** were sensitive to standard reference PM and therefore would be a potentially suitable *in vitro* model of indoor-source PM testing (Vogel et. al., 2005). We also wanted to select a lung-specific cell line, given that this organ represents both the initial entry of PM into the body as well as the area that receives the highest concentration of inhaled toxicants. Due to the diversity of cell types in lung (there are over 40 morphologically different types) a number of different strains were considered:

Table 1-1 Cell lines used in the pilot study

Cell line	ATCC #	Morphology
U937	CRL-1593.2	human monocytes-macrophages
A549	CCL185	human alveolar type II
HPL1	N/A	human peripheral lung epithelial cell
NCI-H441	HTP-174	human Clara cells

ATCC: American Tissue Culture Collection (ATCC, Manassas, VA a repository of culture cells).

A549 are lung cells with characteristics of human alveolar type II cells. These cells are often found at the alveolar septar junction and are responsible for producing and secreting surfactants that reduce alveolar surface tension. **HPL1** cells are non-transformed human peripheral epithelial cells derived by our colleague Dr. Takahashi (Masuda *et al* 1997). **NCI-H441** cells are Clara cell-

derived cells. The main functions of Clara cells are to protect the lung epithelium through detoxification mechanisms and protein secretion. In our pilot studies each of these types were exposed to 2,3,7,8-Tetrachlorodibenzodioxin **(TCDD)**, our control AhR agonist (i.e., a chemical that mimics normally occurring compounds by binding to a cell receptor and triggering a response), urban dust particles **(UDP),** or extracts from **incense** burning. Measures of expression of transcription factors, lung related proteins, markers of inflammation and oxidation were compared among our four test cell lines to determine the best strain to use for our indoor studies.

Chemical Characteristics of Indoor Source PM

For quantitative chemical characterization of indoor source PM, our investigation first focused on PAHs in the PM. PAHs are generated by combustion and the indoor PM sources we investigated all involved heating reactions of carbonaceous compounds. A series of PAHs have been reported in particles generated by cooking activities (Schauer et al., 2002), candle burning (Shi et al., 2007), wood burning (Bari et. al., 2009), and incense burning (Chiang et. al., 2009). Also, some PAHs are known carcinogens and can induce CYP1A1 by activating the aryl hydrocarbon receptor (AhR) (Santodonato et al.,1983). Therefore, these compounds are important to study in conjunction with the biological assays for the indoor PM samples. PAHs were investigated initially by real-time monitoring followed by quantitative chemical analysis. Presence of other compounds were also qualitatively investigated and characterized by gas chromatography/mass spectrometry (GC/MS).

With these approaches in mind, the following objectives were developed for the current study.

Project Objectives

Overall Objective

The overall objective of the proposed study is to develop approaches to assessing the toxicities of PM from several major indoor sources by focusing on the inflammatory and oxidative stress responses of human *in vitro* cell models.

Specific Objectives

1. To evaluate different biological test systems for PM toxicities using indoor PM, outdoor PM, and positive controls and to determine the most sensitive human cell lines for testing a series of indoor PM sources (Pilot Study)

2. Develop and standardize methods for the collection of PM generated by a variety of indoor PM sources (Main Study – goal 1)

3. To evaluate human cell inflammatory and oxidative stress responses to indoor source PM generated during cooking, the burning of candles, the burning of firewood, and the burning of incense. (Main study-goal 2)

4. To incorporate and evaluate the use of bioassay-directed chemical characterization of the most toxic of the indoor source PM samples, and initially chemically characterize the PM. (Main study-goal 3)

2. PILOT STUDY: SELECTION OF TEST CELLS

Objective: To evaluate different biological test systems for PM toxicities using indoor PM, outdoor PM, and positive controls and to determine the most sensitive human cell lines for testing a series of indoor PM sources

2.1 Introduction

Animal testing of the PM generated by different indoor sources would be one approach for toxicity testing, but at considerable cost, time, and use of animal and human resources. Further, the amount of PM samples required for testing may be limiting in such studies. As an alternative, the use of human cell cultures, especially if derived from cells present in the human lung, would be an alternative approach to initially evaluate toxicity from indoor-source PM. Such an approach could also help in chemically characterizing the PM and could help direct investigations of toxic mechanisms of action.

One of the toxic endpoints considered for this project is inflammation since exposure to fine PM is related to systemic inflammation and is a risk factor for cardiovascular diseases (Barnoya and Glantz, 2005). Inflammation, including oxidatively induced inflammation, is hypothesized to be one of the major causes of atherosclerosis and heart diseases (Brook et al., 2003) as well as chronic lung diseases (Hammerschlag et al., 2002).

Tissue inflammatory response, including oxidative stress response triggered by stressors, is not limited to the response of target tissue cells (e.g., epithelial cells) to externally applied stimuli. Rather, it appears to be a result of interactions between mostly blood phagocytotic cells (e.g., macrophages, neutrophils, eosinophils, and dendrite cells) and the target tissue cells. In previous studies, our laboratory investigated the response of macrophages to PM and PM extracts from various sources. In these studies we found that our U937 macrophage model was very sensitive to PM treatment as measured by increased cytokine expression. For this current study, we chose to use this U937 model to investigate cellular responses from a variety of indoor PM sources. In addition, we wanted to select a lung-derived cellular model to emulate possible tissue responses. The goal of the pilot study was to compare the response of different lung cell strains to determine which one is the most sensitive to both indoor- and outdoor-derived PM as well as the AhR agonist TCDD.

2.2 Materials and Methods

2.2.1 Human Cell Cultures

U937 monocytic macrophage cells, A549 lung epithelial cells and NCI H441 lung epithelial cells were obtained from the American Tissue Culture Collection (ATCC, Manassas, VA). HPL-1 immortalized normal human lung epithelial cells were obtained from Professor Takashi Takahashi of Nagoya University, Japan. The cells were maintained as follows:

U937 cells were maintained in Roswell Park Memorial Institute (RPMI) medium 1640 containing 10% fetal bovine serum (FBS; Gemini, Woodland, CA), supplemented with 4.5 g/L glucose, 1 mM sodium pyruvate, and 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid- a buffering agent). Cell cultures were maintained at a cell concentration between 2×10^5 and 2×10^6 cells/ml. For differentiation into macrophages, U937 cells were treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) (3 µg/ml) and allowed to adhere for 48 hr in a 5% CO_2 tissue culture incubator at 37℃, after which they were fed with TPA-free mediu m.

A549 and NCI-H441 lung epiethelial cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. These adherent cells were grown in 10 cm plates and split at 80% confluence.

HPL1 cells were maintained in Ham's F-12 buffer supplemented with 5 µg/ml bovine insulin, 5 µg/ml human transferrin, 10-7 hydrocortisone, 10 ng/ml choleratoxin, 20 ng/ml EGF and antibiotics.

2.2.2 Test Compounds for pilot study

1,2,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was originally obtained from Dow Chemical Co (Midland, MI).

Urban Dust Particles (UDP) were purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). The Standard Reference Material (SRM 1649a) consists of atmospheric particulate material collected in an urban area, and has certified chemical analyses for organic and inorganic compounds.

Diesel Exhaust Particles (DEP) were purchased from NIST as Standard Reference Material (SRM 2975) collected from a diesel fork lift and has certified chemical analyses for organic and inorganic compounds

Incense - Incense was purchased from a major chain store. A variety package was purchased containing 12 incense sticks with wooden cores. Samples for the pilot study took place in a residential bathroom. The PM2.5 cyclone (URG Corp,

Chapel Hill, NC) inlet was positioned approximately 1 m above the incense sticks and the PM was collected at a nominal flow rate of 16.7 Lpm. The sampling time was 20 minutes.

2.2.3 Cell treatment

For screening tests of molecular endpoints, lung epithelial cells or U937 macrophages were treated with 10 µg/ml of standard reference PM, collected indoor particles or TCDD. To reduce the amount of collected particles needed for testing, we minimized our *in vitro* system to 24-well plates containing 5×10^5 cells per well. After 24 hours cells were washed with PBS and prepared for RNA extraction through the addition of RNA extraction lysis buffer.

Cell viability was monitored by the trypan blue exclusion test (McAteer and Davis 1994) for all compounds as follows: A 10-µL portion of re-suspended cell pellet was placed in 190 µL phosphate-buffered saline (PBS) with 200 µL trypan blue (0.5% dilution in 0.85% NaCl) added. After 5 minutes we loaded 10 µL of the cell suspension into a hemocytometer and determined the proportion of nonviable to viable cells.

2.2.4 Methods for Detection of Molecular Markers

RNA extraction

For preparation of total RNA, the cells were homogenized in RNA lysis buffer using a TissueLyser (Qiagen, Valencia, CA). The RNA was extracted with chloroform and further purified with a high pure RNA isolation kit (Qiagen, Valencia, CA).

cDNA synthesis

For quantitative measurement of the mRNA expression level of each marker gene we used the real-time PCR technique. For PCR the RNA was reverse transcribed into the corresponding complementary DNA (cDNA). cDNA synthesis was carried out as previously described (Vogel et al. 2007). Quantitative detection of mRNA expression was performed with a LightCycler Instrument (Roche Diagnostics, Mannheim, Germany) using the Fast Real-Time SYBR Green PCR Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA-free total RNA (1.0 µg) was reverse-transcribed using 4 U Omniscript reverse transcriptase (Qiagen, Valencia, CA) and 1 µg oligo$(dT)_{15}$ in a final volume of 40 µl as described earlier (Vogel et al. 2004).

Quantitative real-time PCR (RT-PCR)

For the specific detection of each marker, we designed primers for each gene on the basis of the respective cDNA or mRNA sequences using OLIGO primer analysis software, provided by Steve Rosen of the Whitehead Institute/MIT Center for Genome

Research. PCR amplification was carried out in a total volume of 20 µl, containing 2 µl of cDNA, 10 µl of 2 × Fast Real-Time SYBR Green PCR Master Mix (Qiagen, Valencia, CA), and 0.2 µM of each primer. The PCR cycling conditions were 95 ℃ for 5 min followed by Two-step cycling 40 cycles of 95 ℃ for 10 s, and 60 ℃ for 30 s. Detection of the fluorescent product was performed at the end of the 60 ℃ combined annealing/extension period. Negative controls were run concomitantly to confirm that the samples were not cross-contaminated. A sample with DNase- and RNase-free water instead of RNA was concurrently examined for each of the reaction units described above. To confirm the amplification specificity, the PCR products were subjected to melting curve analysis. All PCR assays were performed in duplicate or triplicate. The intra-assay variability was < 7%. For quantification, data were analyzed with the LightCycler analysis software according to the manufacturer's instructions.

Data Analysis

Triplicate samples were performed for each experiment. The results represent the mean of three separate experiments and are expressed as fold increases of a treated sample compared to a non-chemical (or solvent only) control. Error bars on the figures represent standard deviation of values used for calculating the mean.

2.3 Test Plan for Pilot Study

The four aforementioned cell lines (U937, A549, HPL1 and NCI-H441) were treated with either the AhR control agonist TCDD, urban dust particles (UDP) or incense-derived particles. After 24 hours, the cells were analyzed by RT-PCR for representative markers of AhR activation (CYP1A1 and IL-8), inflammation (COX-2, IL-8) and lung protein synthesis (Mucin 5AC). The results are summarized on the following pages.

2.3 Results - Pilot Study

Figure 2-1 represents the expression of CYP1A1 in the presence of TCDD, UDP and incense extract. TCDD (top) was able to induce CYP1A1 expression in all samples with U937 being the most sensitive (120 fold) followed by NCI-H441 (48 fold), A549 (16 fold) and HPL-1 (1.6 fold). UDP showed a similar trend but with reduced CYP1A1 expression when compared to TCDD across all cell lines. A549 cells appeared to the most sensitive of the cell strains to incense extract and had a CYP1A1 expression nearly three times greater than with TCDD treatment. The other cell lines had CYP1A1 expressions of about 50% of those seen with TCDD treatment.

Figure 2-2 represents the expression of IL-8 in the presence of TCDD, UDP and incense extract. U937 macrophages appeared to be the most sensitive cell line with the largest increase in IL-8 expression versus control of all the strains tested. Both A549 and HPL-1 had similar IL-8 expression regardless of

the treatment method. NCI-H441 did not appear to express IL-8 in control or treated samples.

Figure 2-3 represents the expression of COX-2 after TCDD, UDP, or incense treatment. Similar to the results observed with CYP1A1 expression, the U937 cells were the most sensitive and had the highest amount COX-2 expression relative to its control. Both TCDD and UDP had similar potency to induce COX-2 in the cell strains, and among the lung cell strains these two treatments elicited the strongest effects on NCI-H441 cells followed by A549 and HPL1 cells. Incense treatment induced COX-2 expression in all strains albeit at lower amounts than seen with other treatments. One exception however, was the A549 cells which showed nearly triple the COX-2 expression with incense extracts that with either TCDD or UDP.

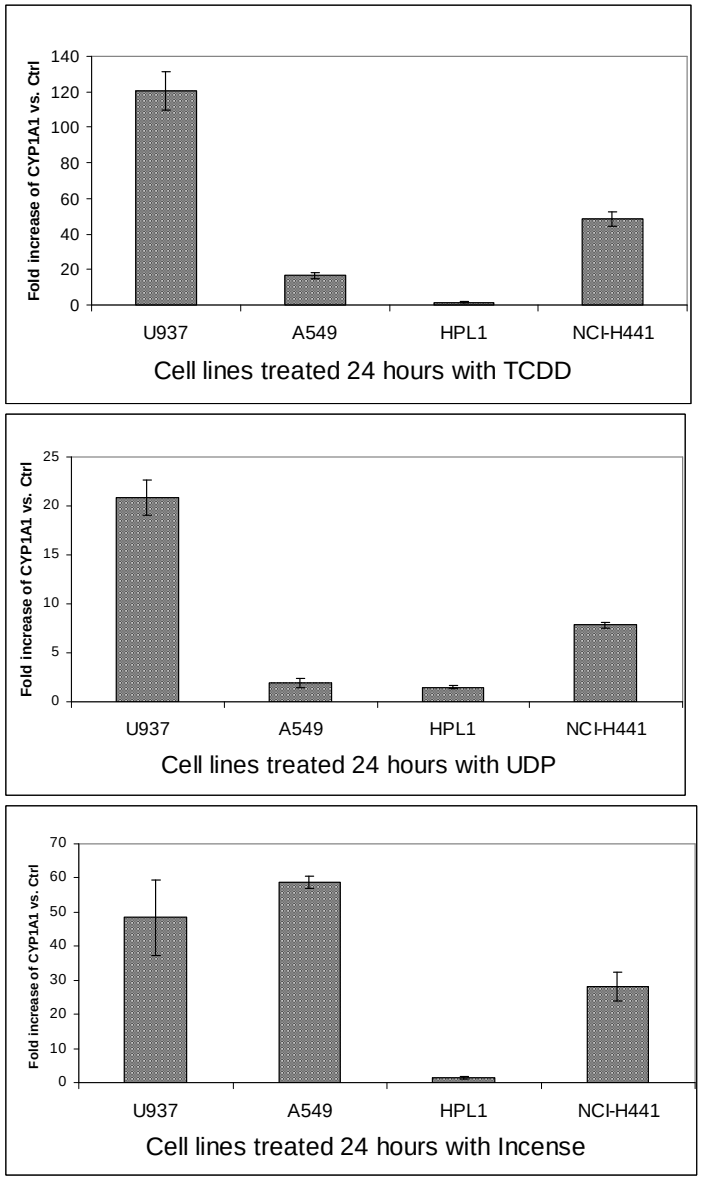


Figure 2-1 Cyp1A1 expression in U937, A549, HPL1 and NCI-H441 cells after 24 hour incubation with TCDD, UDP or incense. Values are expressed as fold increase compared to each cell's respective control. Error bars represent standard errors of the mean.

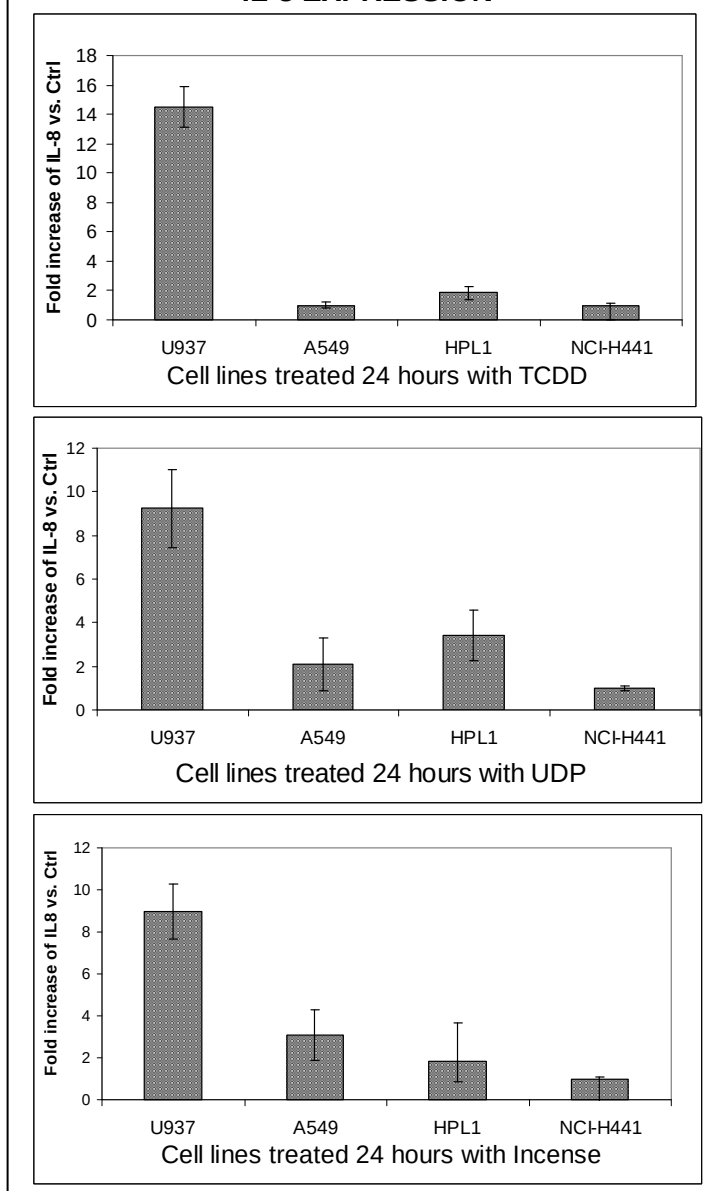


Figure 2-2 IL-8 expression in U937, A549, HPL1 and NCI-H441 cells after 24 hour incubation with TCDD, UDP or incense. Values are expressed as fold increase compared to each cell's respective control. Error bars represent standard errors of the mean.

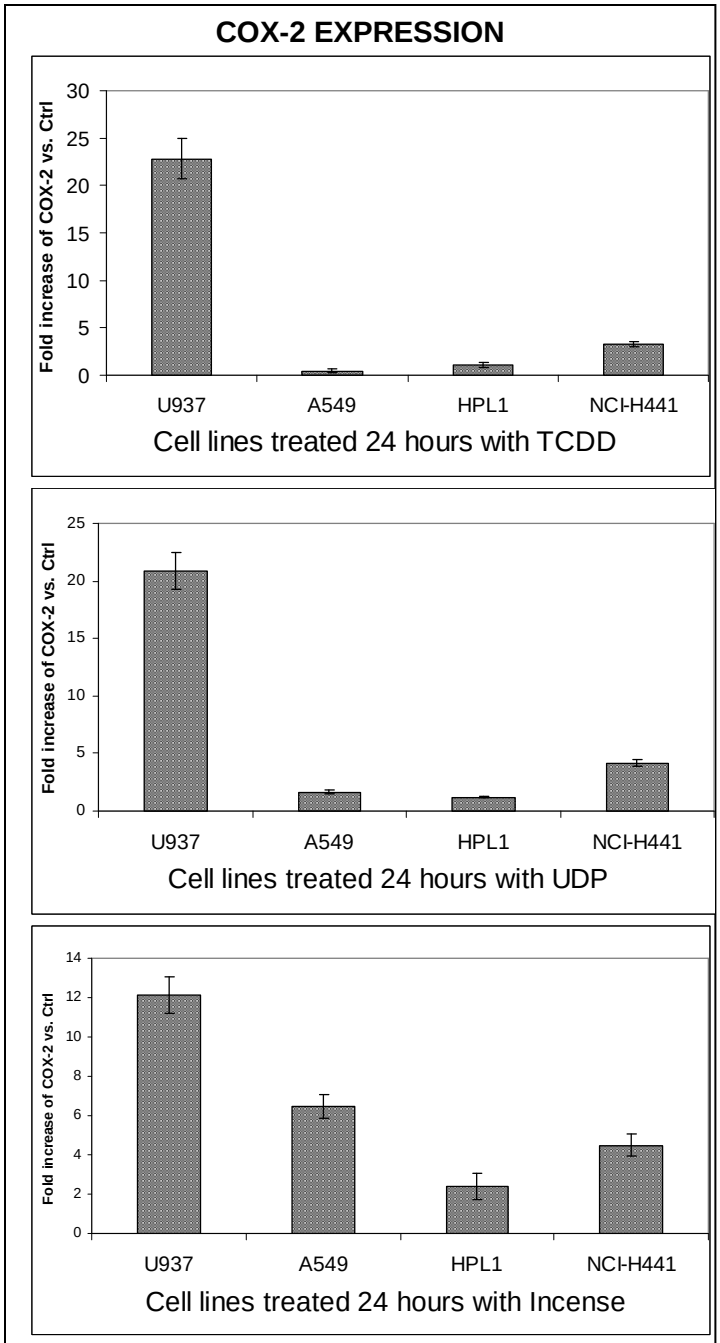


Figure 2-3 COX-2 expression in U937, A549, HPL1 and NCI-H441 cells after 24 hour incubation with TCDD, UDP or incense. Values are expressed as fold increase compared to each cell's respective control. Error bars represent standard errors of the mean.

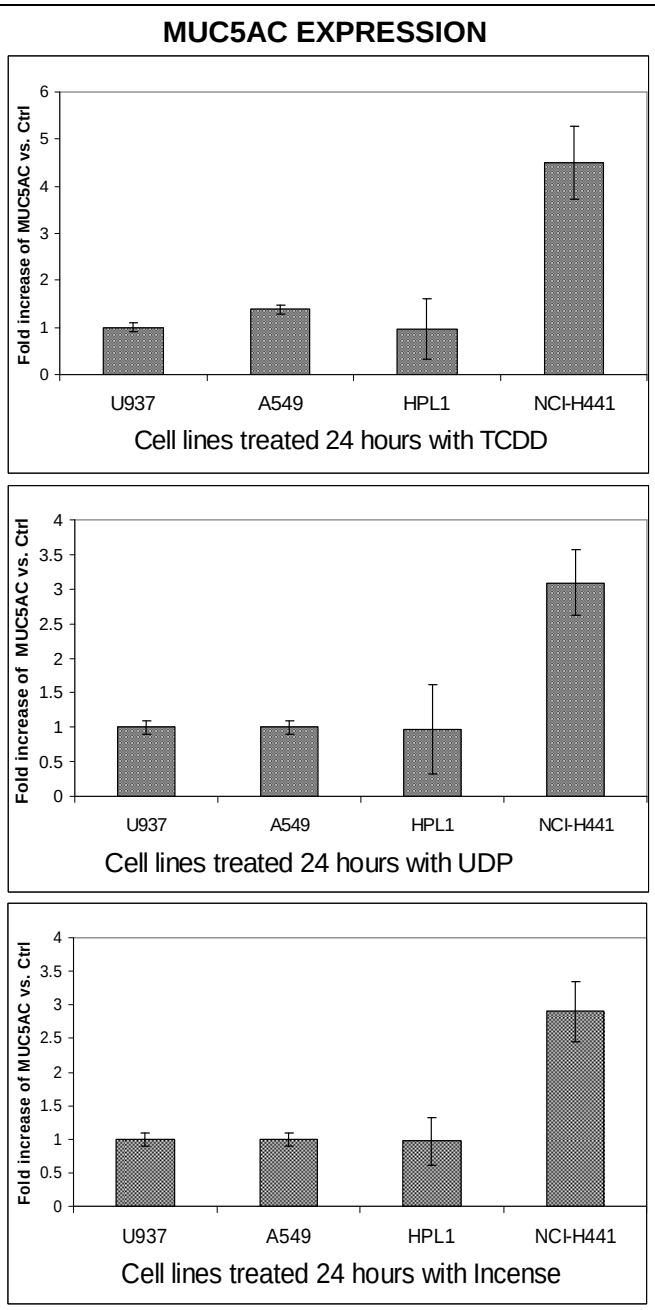


Figure 2-4 MUC5AC expression in U937, A549, HPL1 and NCI-H441 cells after 24 hour incubation with TCDD, UDP or incense. Values are expressed as fold increase compared to each cell's respective control. Error bars represent standard errors of the mean.

Figure 2-4 illustrates the effect of TCDD, UDP, or incense treatment on mucin 5AC (MUC5AC) expression in our test cell lines. This protein appears to be exclusively expressed in the NCI-H441 cell line. The U937 macrophages and HPL1A cells do not appear to appreciably express MUC5AC in either control or treated samples. A549 does express MUC5AC but its expression was not changed by any of the treatment methods. Of the treatment methods TCDD had the greatest effect followed by UDP and then incense extracts.

2.4 PILOT STUDY – DISCUSSION

The U937 macrophage cell line appears to be the most sensitive cell line for investigating changes in cytokine expression of those chosen for the pilot study. Among the lung cell strains, NCI-H441 in general had higher gene expressions for CYP1A1, COX-2 and MUC5AC than A549 and HPL1A. This is not surprising since of the lung-cell lines it has the highest concentration of AhR. NCI-H441, however, did not express IL-8, possibly indicating the lack of a critical molecular component or AhR binding site for this cytokine. Interestingly, A549 demonstrated a stronger effect with incense extract than with TCDD, a pure AhR agonist. This illustrates that incense extracts may be working through a non-receptor mediated pathway in the A549 cellular system or possibly other mechanisms which would potentiate its effects.

2.5 PILOT STUDY - CONCLUSIONS

We feel that the expression of MUC5AC is a key novel finding which our lab has already published (Wong et al., 2010) because it represents a lung-specific protein that appears to be influenced by AhR expression; also, its over-expression has been directly linked to actual health effects (small-airway diseases). Therefore, along with U937, NCI-H441 was chosen as a lung-based cellular model for the main study.

3. MAIN STUDY

Objective: Develop and standardize methods for the collection of PM generated by a variety of indoor PM sources (Main study – goal 1)

Objective: To evaluate human cell inflammatory and oxidative stress responses to indoor source PM generated during cooking, the burning of candles, the burning of firewood, and the burning of incense. (Main study - goal 2)

3.1 Introduction

In the Pre-Test, four human cell systems were evaluated. Two cell systems, the macrophage cell line (U937) and a human lung epithelial cell line (Clara cell; NCI441) were considered the most sensitive for the inflammatory markers based on tests with the standard reference PM samples, positive controls, and selected indoor source PM samples. The next step was to test a variety of indoor source PM and the responses in these cell systems. For this, PM generated during cooking, burning of candles, the burning of firewood, and the burning of incense were tested using these cell systems and the protocols developed for them.

3.2. Materials and Methods

PM Collection

The PM samples collected and analyzed in the human cell culture systems and for chemical analyses are outlined in Table 3-1. PM 10 and PM 2.5 were collected using a cyclone (URG Corp, Chapel Hill, NC) calibrated at a flow rate of 16.7 liters per minute (Lpm). The cyclone is connected to a vacuum pump that has its exhaust ported at least 6 meters away from the collection area usually to the outdoors (for the cooking and woodsmoke samples) or into an exhaust fume hood (for the candles and incense samples). Calibration of flow is conducted prior to and immediately after each sampling period using a DryCal DC-Lite (Bios International, Butler, NJ) calibrated primary standard flow calibration device. The cooking and woodsmoke samples were obtained in the field in residences. The candles and incense samples were collected in the laboratory setting so that numerous samples could be collected, sampling devices could be conveniently solvent cleaned between samples, and candle and incense odors and exhaust could be vented conveniently. Details of sampling for the respective indoor source samples are detailed below.

Selected vapor-phase samples at least one each for cooking, candles, woodsmoke and incense were collected using XAD adsorbent (XAD-2) placed in series behind the filter cartridge and were analyzed for the chemical analyses phase of this project (Section 4).

Quantitative Chemical Analyses of PAHs

Quantitative PAH analyses were conducted for extracts from the indoor PM source samples. Gas chromatography/mass spectral (GC/MS) methods previously published for diesel and heavy-duty engine emission exhaust PM (Okamoto et al., 2006; Kado et al., 2005) were employed. Briefly, a Hewlett-Packard (HP) 5890 Series II gas chromatograph interfaced to a HP5972 mass selective detector run in selective ion monitoring mode (SIM) was used throughout. The injector was operated in splitless mode. The GC was equipped with a DB-5ms fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). PAH standard reference material SRM 2260 (NIST, Gaithersburg, MD) was used to prepare calibration solutions. Additionally, a limited number of XAD samples were also analyzed for PAHs. Briefly, XAD samples were extracted in DCM four times by shaking in separatory funnels. To remove interfering compounds, extracts were subjected to silica fractionation to isolate the PAH fraction.

Real-Time PAH survey of the Indoor Source PM samples

An initial chemical survey was conducted using the EcoChem PAS PAH sampler (Ecochem Analytics, League City, TX) to see if particle-associated PAHs could be detected in selected samples. The instrument was kindly loaned to us for this purpose by the manufacturer. The PAS instrument detects the PAHs associated with the particles and reports the concentrations in nanograms (ng)/m^3. Typically, the instrument is sensitive to PAH that have 3 or more rings. (PAHs are fused benzene rings). An example of a PAH associated with PM with 3 rings is phenanthrene.

Qualitative Chemical Characterization of the Indoor PM source samples

Qualitative GC/MS scans of the indoor source extracts were performed to provide general chemical characterizations of the indoor source PM samples.. These total ion current chromatogram (TIC) GC/MS scans (general scans) were conducted in the electron impact mode using a Hewlett-Packard (HP) 5890 Series II gas chromatograph interfaced to a HP5972. The GC was equipped with a DB-5ms fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness).

The mass spectrum for major peaks were compared with the integrated National Institute of Standards and Technology (NIST, Gaithersburg, MD) library to identify the compounds. The extracts investigated were the same ones that were tested by the biological tests. The PM equivalents used for the TIC analyses depended on the PM amounts collected, therefore they were different among different samples.

Testing in Human Cells

A spectrum of inflammation and oxidative responses and the test matrix of indoor-source PM samples tested are summarized in Table 3-2 for the human macrophage cells and for the human Clara cells. The macrophage cells do not produce mucin, while the Clara cells do produce mucin. In each of the cells indoor PM source samples were tested (n): number of samples. The procedures detailed in the Pilot Study are used in the Main Study. The amount of sample added for the experiments was at a 10 µg of PM "equivalent". For this, a volume of extract equivalent to 10 µg of PM is added per ml of cell culture in Dimethyl Sulfoxide (DMSO) to aid adding the extract to the aqueous cell culture medium. The level of DMSO was typically less than 0.1% total volume. The level of PM provided a balance between providing adequate amounts of material versus the amount of PM collected for many of the samples.

Table 3-1 PM samples collected and tested

Indoor Source PM Collected	**PM Samples and Analyses**
Cooking (2)	PM 10 samples for cell testing and chemical analyses.
Candles (6)	PM 2.5 samples for cell testing and chemical analyses.
Wood burning (4)	PM 2.5 and PM10 samples for cell testing and chemical analyses.
Incense (6)	PM 2.5 samples for cell testing and chemical analyses.

(n) number of samples

Table 3-2. Cell Response markers measured in the indoor source PM

	Macrophage (U937) and Clara Cells (NCI H441)		Macrophage only	Clara Cell only
Indoor PM Source Samples	**Xenobiotic Enzyme Receptor (CYP1A1)**	**Inflammation Prostaglandin (COX-2)**	**Inflammation/ Cell Recruitment (IL-8)**	**Mucin Production (MUC5AC)**
Cooking (2)	**2**	**2**	**2**	**2**
Candles (6)	**6**	**6**	**6**	**6**
Wood burning (4)	**4**	**4**	**4**	**4**
Incense (6)	**6**	**6**	**6**	**6**

( ) = number of samples tested.

3.2.1 Cooking

Introduction

The PM samples from cooking were obtained from stir frying and from oven baking based on some of the highest PM emissions reported by Fortmann and colleagues (2001). Buonanno et al. (2009) reported that certain cooking procedures such as frying produced PM in the size range of 0.006 to 20 µm. We followed cooking procedures typically used in the home. A PM 10 size cut was therefore used during the cooking event. Cooking was performed using published recipes following routine procedures with food items that were readily available at the market. Cooking was performed with a single event cooking protocol that was repeated during a one-day test period. New oil poured from the bottle was used for every single event. We followed cooking procedures typically used in the home.

Stir-Fry

The stir-fry procedure was conducted on a gas stove (Modern Maid) equipped with four burners. The cooktop was a 30 inch wide porcelain-on-steel surface with open gas burners (9,000 Btu max). The sampler inlet was placed approximately 30 cm above the cooking surface which was an approximate height of the cook's breathing zone, and was not at a location where heat would be a factor in the collection. Before any cooking was initiated, a background air sample was collected. The background sample was handled in an identical manner as the sample for cooking, except the gas burner was not turned on. Samples were collected at a flow rate of 16.7 Lpm for 60 min using Teflon filters (Zefluor, Pall Corp, Port Washington, NY) and XAD adsorbents. The CO, CO_2, temperature, and relative humidity of the air near the sample inlet were monitored throughout the sampling using the Q-Trak instrument (TSP Inc. St. Paul, MN). After taking the background air sample, the source PM sample was a Chinese style stir-fry consisting of chicken meat, vegetables (onion, garlic, green onion, sugar peas, cabbage, ginger, bell pepper) and seasoning (soy sauce, sugar, and cornstarch). A new wok (carbon steel, 12 in diameter) heated over gas flame was used throughout the cooking. The ingredients used and amounts are summarized in Table 3-3. The wok was washed with dishwashing detergent, rinsed, dried, and pre-seasoned to condition it before any food was added by repeated heating with approximately ¼ C peanut oil (repeated 3 times).

The sampling inlet was placed approximately 30 cm above the wok as seen in Figure 3-1. Also, the temperature of the cooked food was monitored using a chromel-alumel thermocouple that was placed inside the wok during the cooking, and the wok temperature was recorded using a data logger. The sampling was repeated four times with air sampling. When one batch of cooking was completed after 20 min. (Stir-fry 1), the sampling was stopped. All cooking

utensils were cleaned by washing in dishwashing detergent, and the 2nd stir-fry event cooking event was started (Stir-fry 2). PM 10 samples were collected back-to-back on a single Teflon filter representing a composite sample of stir-fry 1 and 2. A vapor-phase cartridge consisting of XAD for the chemical analyses was placed in series behind the filter. Stir-fry trial 3 and 4 were each approximately 20 min in cooking time and the PM10 was collected as back-to-back samples on a single Teflon filter. A vapor-phase XAD sample was also collected in series for stir-fry 3 and 4. The maximum wok temperature reached during the stir-fry any of the cooking was 286 ºC.

Table 3-3 Stir-Fry Ingredients and Amounts Used

Ingredients	Ingredient Wet Weight (g)				Description
	Stir-Fry 1	Stir-Fry 2	Stir-Fry 3	Stir-Fry 4	
	CK-1		CK-2		
Chicken breast	509.1	512.4	493.8	502.8	White meat separated from bones. Foster Farms fresh
Garlic	20.2	22.8	12.3	12.9	6 cloves, Fresh, chopped
Ginger	6.37	7.11	5.04	5.76	Fresh, grated
Onion	173.36	141.38	126.24	124.86	1 onion, Fresh sweet white, diced into small cubes
Green onion	24.37	32.96	30.299	24.52	Chopped
Sugar snap peas	175.96	160.08	170.73	163.74	Fresh, whole
Cabbage	55.96	45.32	47.56	53.41	Fresh, sliced
Bell pepper	73.71	79.73	107.35	106.73	Fresh, sliced
Peanut oil	¼ C x 2	¼ C x 2	¼ C x 2	¼ C x 2	Planters
Sauce					
Soy sauce	2T	2T	2T	2T	Kikkoman, regular
Sugar	2T	2T	2T	2T	
Cornstarch	2T	2T	2T	2T	Kingsford’s
Water	1/2 C	1/2 C	1/2 C	1/2 C	

T = Tablespoon (approximately 15 ml)
C= Cup (approximately 240 ml)

Figure 3-1 Stir-fry and sampling set up

Oven Cooking

PM samples were also collected from oven cooking. Chicken meat seasoned with teriyaki sauce, soy sauce, and ginger was cooked in an electric oven (GE Appliances model 371G) at 350 ºF (177 ºC). The recipe is summarized in Table 3-4. PM and vapor-phase samples were taken at 16.7 Lpm for 66 min from beginning to the end of cooking chicken. The sample inlet was placed near the oven vent, located approximately 11 cm above and 10 cm horizontally spaced from the oven, as seen in Figure 3-2. CO, CO_2, temperature and relative humidity of the air near the sample inlet were monitored throughout the sampling. PM samples were post-weighed to obtain sampling mass.

Baked Teriyaki Chicken

Table 3-4 Ingredients for the baked teriyaki chicken PM sample

Ingredient	Amount of Ingredient	Description
Chicken thighs bone-in	2.04 Lb	Dark meat with bone, Foster Farms fresh
Ginger	5 slices	5 Slices
Soy sauce	¼ C	Kikkoman regular
Brown sugar	2.5 T	Light brown C & H
Water	¼ C	Tap water

T = Tablespoon (approximately 15 ml)
C=Cup (approximately 240 ml)
Lb = pound (approximately 454 grams)

Oven temperature = 350 ℉ (177 ℃)

Cooking procedure:

Pre-heat oven to 350°F (177°C). Mix soy sauce, ginger, brown sugar, and water to make sauce in a cup. Add chicken to 9 X 13 inches Pyrex baking pan skin side up. Pour sauce over chicken. Bake 1 hr 15 min at 350 ℉.

Air sampling procedure:

Prior to placing the chicken in the oven, the PM10 sampling head was positioned approximately 6 inches (15 cm) above and 1 foot (30 cm) away from the oven vent. The sampler inlet was positioned above the oven, near the vent, as seen in Figure 3-2. The inlet was selected to maximize the collection of PM sample. The oven was pre-heated to 350 ℉ (177°C) prior to adding the chicken. As soon as the chicken was placed in the oven and the door closed, sampling was initiated.

Figure 3-2 sampling of oven emissions from baking chicken

3.2.2 Candles

3.2.2.1 Introduction

According to the National Candle Association (NCA, 2010) candles are used in 7 out of 10 U.S. households with $2 billion annual sales excluding accessories. The NCA also reported that approximately 1 billion pounds of wax is used in producing candles sold in the U.S. annually. The particle size reported for candle PM has been in the range of 20 to 100 nm (Li and Hopke, 1993) to 100 to 800 nm (Fine et al., 1999). Since candles appear to be widely used consumer products that can be used indoors, they were tested as a source of PM.

3.2.2.2 Materials and Methods

Candles were purchased from various retail stores and represented a variety of manufacturing countries (Table 3-5). The stores were major retail outlets and are coded. For collecting PM from a variety of candles, a laboratory sampling setup was developed. These products produce PM, heat, and are very aromatic, some with intense and persistent odor. Therefore, a location was established where multiple samples could be taken, sampling equipment set up, and the candle or incense sample emissions tested without perturbing the conditions of combustion. The sampler and candle was placed on a platform or small table near a chemical fume hood. The candle was placed so that the flame remained vertical and the emission gently trailed upward and then toward the hood (Figures 3-3 and 3-4). The cyclone was placed approximately 1/3 m above and 1/3 m downwind from the candle plume to allow the emissions to pass near the sampler inlet. This position was considered optimum for PM and allowed the emissions to gently flow to the hood. The candles were lit with a butane fueled hand-held (pistol grip type) lighter with a lighting time of nominally < 2 sec. Candles were new and sampling was initiated immediately after the wick started to maintain flame. The total sampling time was 60 min and candles were blown out at 20 and 40 min and relit after 5 sec each time. At the 60 min time, the candle was blownout without relighting.)

Filters used for these studies were 47 mm Teflon (Zefluor, Pall Corp, Port Washington, NY) pre-cleaned with methanol (3 times), followed by Dichloromethane (DCM) (3 times) with shaking. Filters were dried in a HEPA-equipped laminar flow hood for at least 24 hr. Filters were pre-weighed in a temperature-humidity monitored room using a microbalance; Cahn Model 31 (Thermo Fisher Scientific, Waltham, MA). Filters were extracted using DCM with shaking, followed by sonication (Bransonic model 5510; Danbury, CT) for 15 min each time. The temperature in the sonication bath was maintained nominally near 25°C. The procedure was repeated 3 times. Th e extract was concentrated

by removing most of the DCM under a steady stream of nitrogen. The final volume of extract was approximately 0.5 ml and transferred to 1 dram pre-cleaned amber vials. The extracts in DCM were designated “stock” solutions for biological analyses. For use in bioassay analyses, aliquots of this stock were transferred into ½ dram amber glass vials that were pre-cleaned with acetone and baked at 550℃ for 8 hr. The DCM extracts were dried under a stream of nitrogen, and re-suspended in DMSO. Very low levels of DMSO with the extract could then be added to the aqueous incubation mixture used for experiments.

Table 3- 5 Candles acquired and tested in bioassay.

Candle ID	Name	Characteristics	Color/Style	Size	Store	Manufactured In
A	Tindra Ljuv	Scented Candle	Red, Filled Glass	2.5" dia X 1.8"	2	China
B	3" Fresh Cotton	Scented Candle	White, Pillar	2 3/4" (D) X 3"(H)	3	India
C	Botanica Candles	Scented and Handcrafted Candle, Mango Papaya	Orange Red, Pillar	260 g, 2 7/8" (D) X 3" (H)	4	Hong Kong
D	Paula Deen	Scented Candle, Pear Honey	Green, Filled Glass (Container)	16 oz (453 g)	1	USA
E	Scented Gold Ring Pink	Scented Gold Ring, Religious	Flamingo, Filled glass	2 1/4" (D) x 8 " (H)	2	USA
F	Renew	Hand poured, Jasmine & Tea Leaf	Coral, Pillar	8.8 oz/250 g, 2.75" x 3 "	4	Vietnam

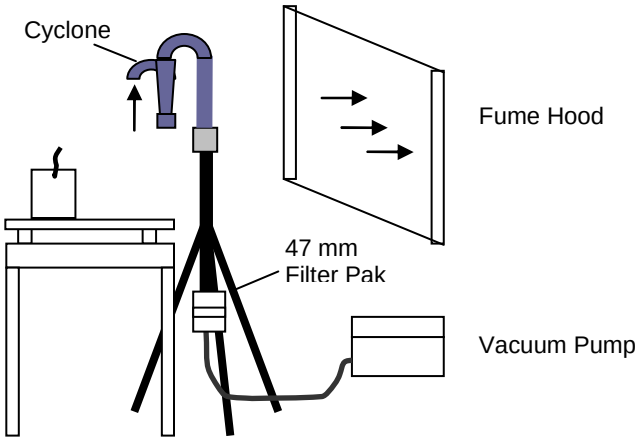


Figure 3-3 Diagram of candle PM sampling apparatus setup.

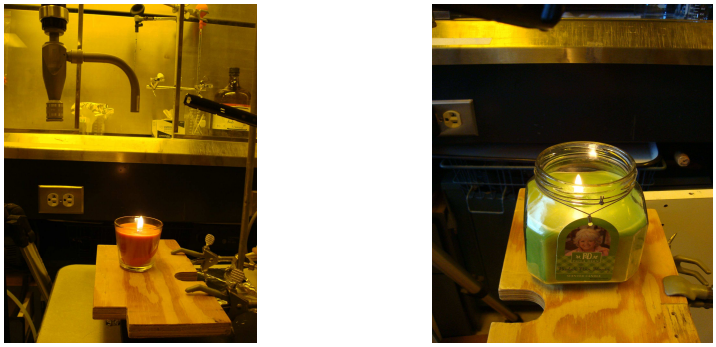


Figure 3-4 Samples of candle burning and sampling.
Vertical flame and plume of PM directed toward sampling head.

3.2.3 Woodsmoke

3.2.3.1 Introduction

Woodsmoke is a complex mixture of PM and vapor-phase components that have been reported indoors with many of the identified compounds reported as toxic air pollutants (Zelikoff et al, 2002). The particle sizes that have been reported are generally smaller than 1 µm and range between 0.15 and 0.4 µm (Hayes et al., 2002).

3.2.3.2 Materials and Methods

The sampling of indoor near-source woodsmoke PM was conducted at a local residence equipped with a fireplace. Aged almond firewood acquired locally near Davis, California was used throughout. The fireplace was constructed of red brick and measured nominally at the opening 1 m in width x 0.5 m in height. The damper was in its normal open position. PM2.5 and PM10 samplers were positioned approximately 1.0 m away from the fireplace opening as diagramed in Figure 3-5 and viewed in Figure 3-6. The samplers were positioned as close as possible to the fireplace, but the 1 m distance was considered optimum since any closer resulted in samplers becoming hot. We were concerned about passing the hot emissions over the PM which could result in loss of semi-volatile compounds. The PM was collected on Teflon filters (Teflo, Pall Corp, Port Washington, NY). A real-time PAH monitoring device (PAS 2000, Ecochem Analytics, League City, TX) that measures PM associated PAHs was used during the sampling. The instrument was kindly loaned to us by Ecochem Analytics and works on the principle of photoionization of particle-bound PAHs. It can detect PAHs with 3 or more rings in the nanogram per m^3 range. An XAD cartridge was placed in series with the PM samplers for chemical analyses. Sampling time for each set of PM samples was approximately 45 min. Two sets of the burning of firewood were conducted labeled as WS-1 (Burn 1) and WS-2 (Burn 2).

Filters used for these studies were 47 mm Teflon (Teflo, Pall Corp, Port Washington, NY) pre-cleaned with methanol (3 times) with shaking. Filters were dried in a HEPA-equipped laminar flow hood for at least 24 hr. and were pre-weighed in a temperature-humidity monitored room using a microbalance; Cahn Model 31 (Thermo Fisher Scientific, Waltham, MA). After sampling, the Teflon filters were cut away from the polymethylpentene ring and the filter extracted using DCM, first with shaking for 15 min., followed by sonication (Bransonic model 5510; Danbury, CT) for 15 min. The procedure was repeated 3 times. After each sequence of shaking and sonication, the resulting solvent was transferred to a 50 ml Turbo Vap tube, and the DCM was evaporated under a

steady stream of nitrogen. The volume of extract was evaporated to an approximate final volume of 0.5 ml and transferred to 1 dram pre-cleaned amber vials. The extracts in DCM were designated “stock” solutions for biological analyses. Aliquots of this stock were transferred into 1 dram amber glass vials, and this “working solution” was dried under a stream of nitrogen, and re-suspended in DMSO. Very low levels of DMSO (less than 0.5%) containing the extract are added to the aqueous incubation mixture used for the human cell experiments.

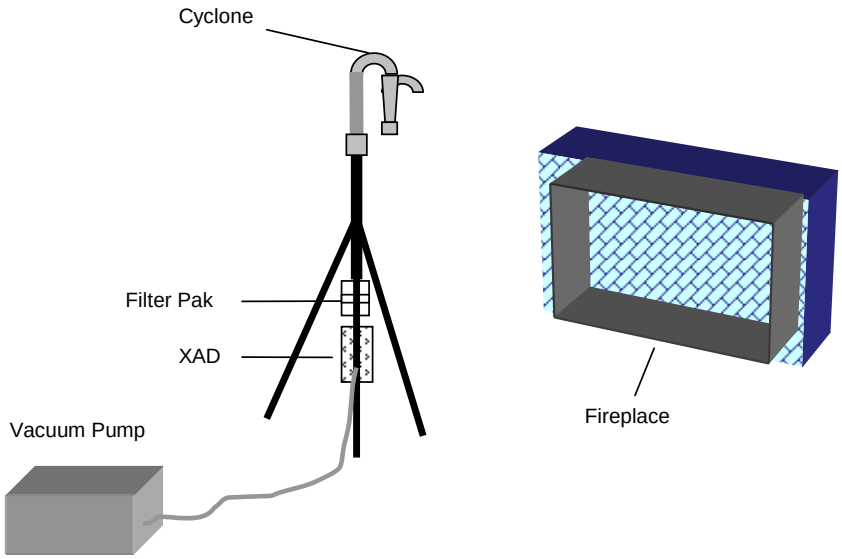


Figure 3-5 Diagram of the sampling setup for woodsmoke.
Sampler placed approximately 1 m from fireplace opening.

Figure 3-6 Sampling setup for woodsmoke.

3.2.4 Incense

3.2.4.1 Introduction

Incense is made from a variety of products including resins, woods, gums, and charcoal. This material is made into a paste with water and can be wrapped around a wood core or stick (U.S. EPA, 2001). Regarding PM size anticipated, Mannix et al. (1996) estimated that the mass median diameter of incense smoke was between 0.24 and 0.40 µm. Li and Hopke (1993) reported that incense burning produced particles in the size range of 0.1 to 0.7 µm. We therefore focused our sampling to PM2.5.

A variety of incense samples was obtained from major stores or mail order as summarized in Table 3-6.

For collecting PM from a variety of incense samples, a laboratory sampling setup similar to that developed for candles testing in a laboratory setting was developed, except filter size was increased from 47 mm to 70 mm so that sufficient PM would be collected without overloading the filter media. Briefly, 70 mm Teflon filters (Zefluor, Pall Corp, Port Washington, NY) were pre-cleaned with methanol and DCM (3 times) with shaking. Filters were dried in a HEPA filtered 100% exhaust hood for a minimum of 24 hr.

For the sampling, the flow rate was maintained at 16.7 Lpm using the PM2.5 cyclone sampling head. Flow rates were determined before sampling and immediately after the sampling period using a Dry Cal Flow meter. PM 2.5 was selected since there are a number of reports indicating PM size for incense is less than PM 2.5 (Mannix et al., 1996; Li and Hopke, 1993). The incense once lit had very noticeable smoke as well as persistent aromas and odors. The approach used for candle sampling in the laboratory was used for the incense. In this manner, the sample could be reproducibly obtained, and the emissions would be exhausted without perturbing the conditions of combustion (Figures 3-7 and 3-8). The cyclone was positioned approximately 1/3 m above and 1/3 m downwind from the burning tip of the incense. This placement allowed the plume of the emission to flow freely across the inlet. The emissions would eventually vent into the hood. To provide some information regarding the burn rate of the incense, incense sticks were pre-weighed individually, and post-weighed after burning the incense.

Table 3-6 Incense samples acquired and tested for bioassay

Sample ID	Name	Color	Size	Type	Store	Manufactured
A	Nag Champa	Brown	15g Net weight	Wood core	1	Bangalore, India
B	Pure Tibetan- Herbal Medicine	Brown	N/A	No Core	1	Kathmandu, Nepal
C	Shoyeido Traditional Japanese	Multi	.017 oz per stick, 10 sticks/Pack	No Core	1	Kyoto, Japan
D	Pure Tibetan- Potala	Red	N/A	No Core	1	Nepal
E	Aromatherapy variety	Multi	10 in, 24 sticks/Pack	Wood core	2	Mumbai, India
F	Joss Sticks Mainichikoh	Green	107 sticks	No Core	3	Japan
G	Floral variety	Multi	10 in, 24 sticks/Pack	Wood core	2	Mumbai, India

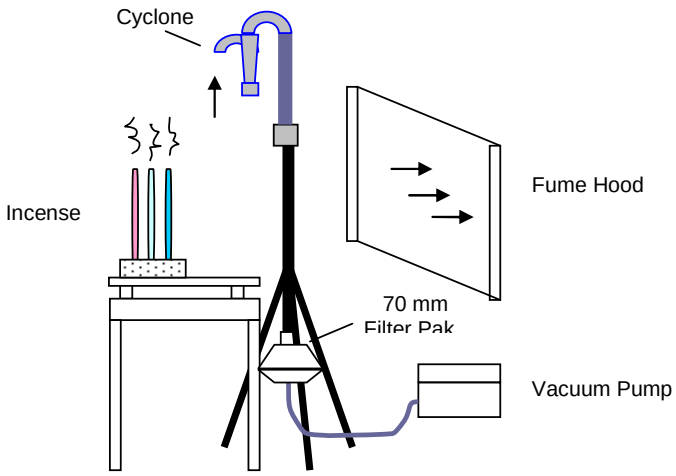
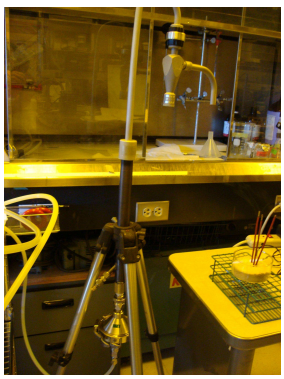


Figure 3-7 Diagram of the sampling setup for incense



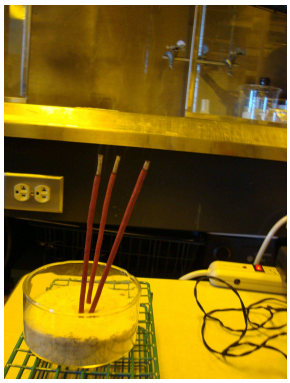


Figure 3-8 Sampling set-up for incense PM sampling

3.3 RESULTS MAIN STUDY

3.3.1 Introduction

The indoor PM source samples were tested using the human cell assay system where particles are first trapped by filter, extracted by organic solvent, and then introduced into the *in vitro* human cell culture system described. A number of markers for inflammation and a marker for oxidative stress were measured. In this Results section, we detail the results for the indoor PM source samples from cooking, candle burning, wood burning, and incense burning. These samples were tested in human macrophage cells (U937) and in human lung cells (Clara like cells; NCI H441).

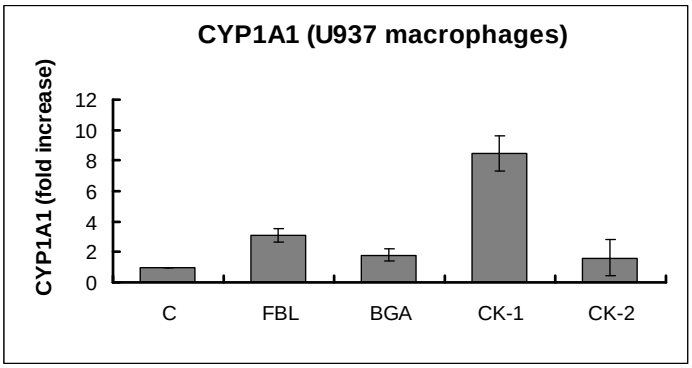
3.3.2 Cooking PM

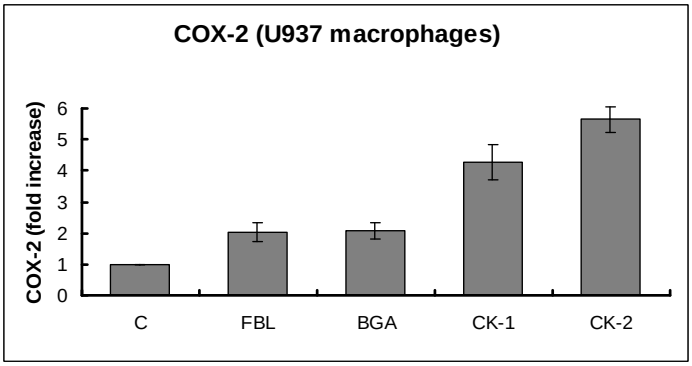
The PM mass measurements for indoor cooking samples varied according to sampling parameters. The stir-fry samples represented back-to-back samples, with an average PM10 mass of 7.861 mg/filter collected for a total of 40 min, with an ambient mass (background) of 9 µg/filter collected for 60 min. The oven sample was considerably lower than the stir-fry samples, with a PM10 mass of 77 µg/filter collected for 66 min. The PM10 mass values are summarized in Table 3-7.

Table 3-7 PM10 mass measurements of cooking samples.

Sample	ID	PM10 Mass (mg/filter)	Adsorbent for Vapor Phase (for Chemical Analyses
Stir-Fry Trial 1 & 2	**CK-1a**	**6.212**	**X**
Stir-Fry Trial 3 & 4	**CK-1b**	**9.509**	**X**
Oven cooking	**CK-2**	**0.077**	**X**
Background Air	**BGA**	**0.009**	**X**

Indoor PM samples from cooking (stir-fry and oven cooking) were tested in U937 human macrophages and the NCI H441 human lung cell line (Clara cells) for relevant biological markers of PM toxicity. The cells were treated for 24 hr with 10 µg/ml particle-equivalent organic extract. The mRNA expression of CYP1A1, COX-2, IL-8 and MUC5AC was analyzed using real-time PCR. Figures 3-9 and 3-10 illustrate cells treated with the indoor PM from cooking sources and the response of the human macrophages U937 and NCI H441 human lung cell lines, respectively.





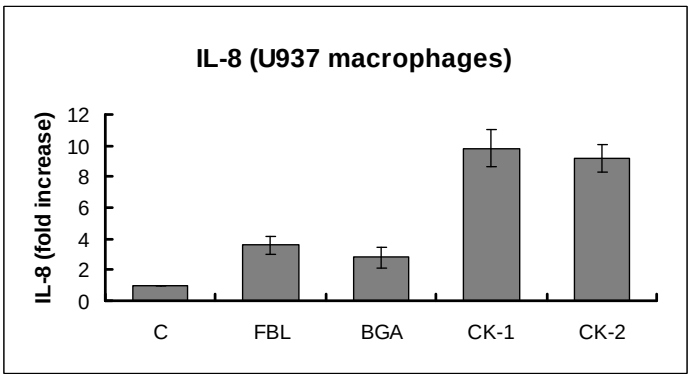
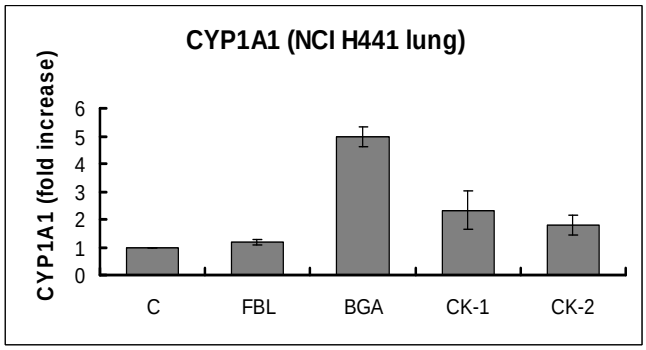
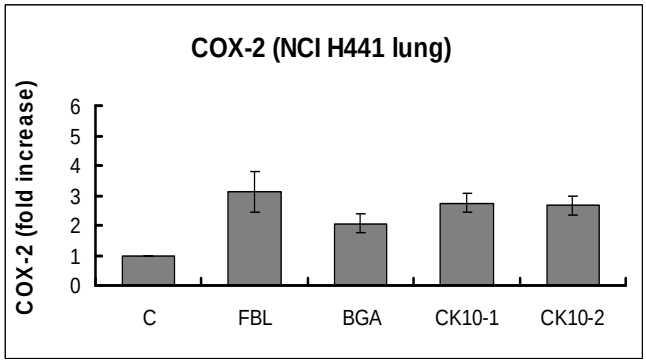


Fig 3-9 Effect of cooking source samples on CYP1A, COX-2 and IL-8 mRNA expression in U937 macrophages

Cells were treated for 24 hr with 10 µg/ml PM equivalent (organic extract) from cooking source PM samples. Error bars represent mean ±SD of triplicate determinations.

C: Vehicle control, FBL-2: Field Filter Blank, BGA: Background Air PM10 filter extract, background CK-1:Stir-fry cooking, CK-2: Oven cooking





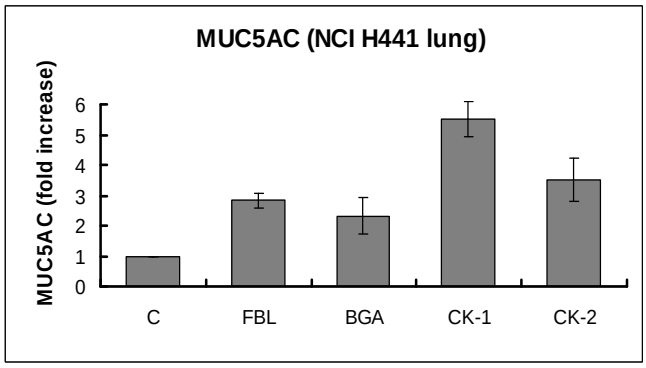


Fig 3-10 Effect of cooking source samples on CYP1A, COX-2 and MUC 5AC mRNA expression in NCI H441 lung cells

Cells were treated for 24 hr with 10 µg/ml PM equivalent (organic extract) from cooking source PM samples. Error bars represent mean ±SD of triplicate determinations.

C: Vehicle control, FBL-2: Field Filter Blank, BGA: Background Air PM10 filter extract, background CK-1:Stir-fry cooking, CK-2: Oven cooking

The most significant effect on CYP1A1 expression in macrophages was observed after treatment with stir-fry cooking PM (Figure 3-9 top). The oven-cooking PM had no significant effect on CYP1A1 mRNA level. On the other hand, both cooking samples from stir-fry and oven cooking increased inflammatory marker COX-2 (Figures 3-9 and 3-10, middle) as well as IL-8 (Figure 3-9, bottom) in macrophages. The PM extract from oven cooking was slightly more potent than PM extract from stir-fry cooking for COX-2 in macrophages. For the NCI-H441 cells, the expression of CYP1A1 or COX-2 was not significantly changed by treatment with extracts from cooking PM. The only significant effect in NCI H441 cells was found on MUC5AC expression by treatment with extract from the stir-fry cooking sample (Figure 3-10 bottom).

3.3.2.1 Summary

Besides a slight effect of oven cooking PM on COX-2 and IL-8 in U937 macrophages only the extract of the stir-fry cooking PM generated significant effects on all markers tested (CYP1A1, COX-2, IL-8 in macrophages, and MUC5AC in macrophages and NCI H441 cells). Higher concentrations and different cooking conditions should be tested to identify a potential toxic or inflammatory effect.

3.3.3 Candle PM

3.3.3.1 Introduction

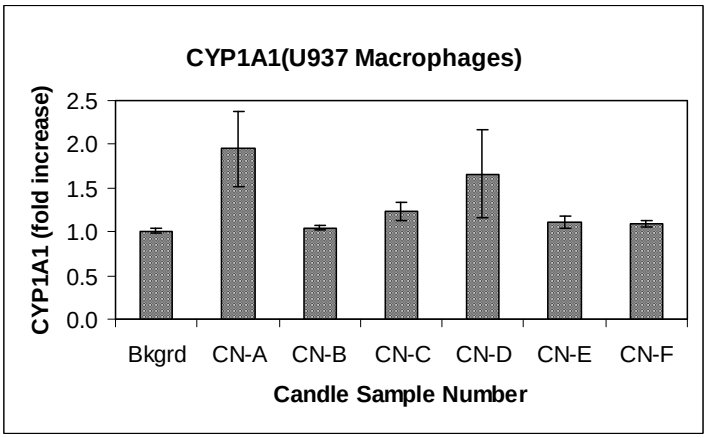
Besides cooking (broiling, frying, etc), candles have been reported to be an additional important source of indoor PM. For instance, a citronella candle had been found to be an extremely powerful PAH source (Wallace 2000). As mentioned in the Methods section, according to the National Candle Association (NCA, 2010) candles are used in 7 out of 10 U.S. households, with estimated annual sales of $2 billion, excluding accessories. The main types of candles sold include pillar type (cylindrical in shape) and container type (the candle is formed in a container usually glass). In the current study a number of these candle types were screened.

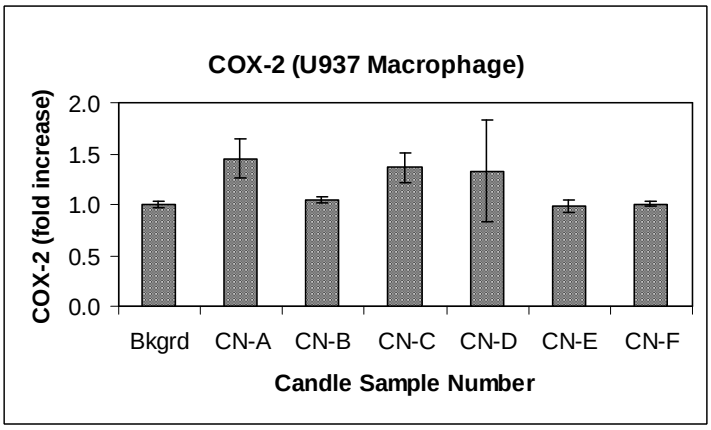
3.3.3.2 Results

We exposed the sensitive U937 macrophages to 10 μg/ml PM-equivalent organic extract from burning different candles (CN-A through CN-F).

Table 3-8. PM mass measurements of candle samples.

Sample	ID	PM 2.5 Mass (mg/filter)
Candle Scented Container Type	**CN-A**	**2.473**
Candle Scented PIllar Type	**CN-B**	**0.162**
Scented Pillar Type	**CN-C**	**2.594**
Scented Container Type	**CN-D**	**0.645**
Scented Container Type	**CN-E**	**0.182**
Scented Pillar Type	**CN-F**	**0.878**
Background Air	**BGA**	**0.001**





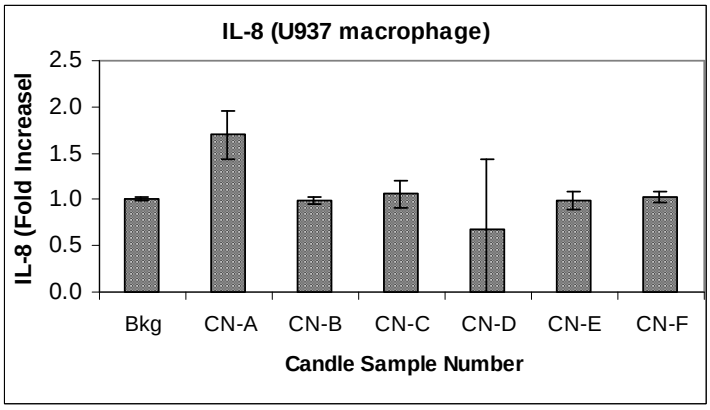
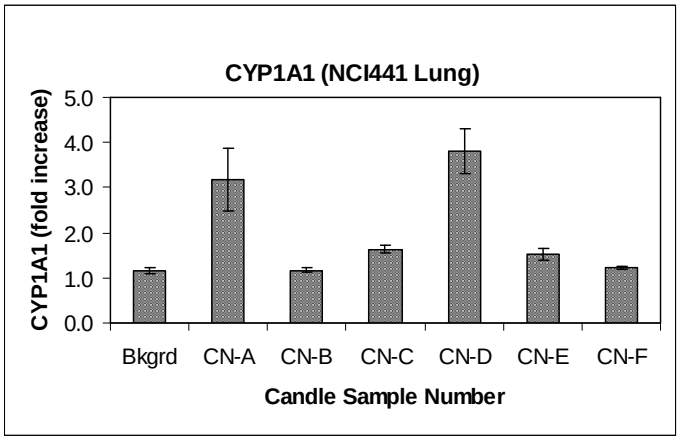
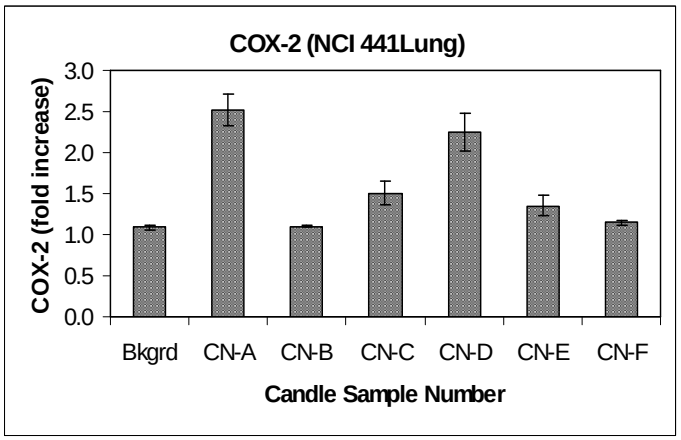


Figure 3-11 Effect of candle indoor-source PM samples on mRNA expression in U937 macrophages

Cells were treated for 24 hr with 10 µg/ml PM sample used per determination from the organic extract. Background air (Bkg) was included as a control. Candle samples are labeled CN-A to CN-F. Error bars represent mean ±SD of triplicate determinations.





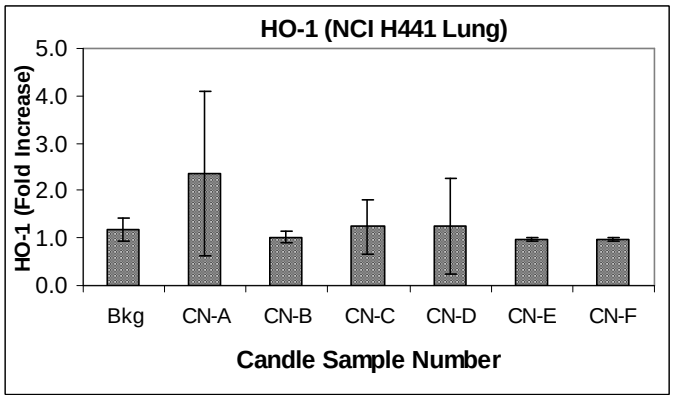


Figure 3-12 Effect of candle indoor-source PM samples on mRNA expression in NCI H441 cells.

Cells were treated for 24 hr with 10 µg/ml PM sample per determination from the organic extract. Background air (Bkg) was included as a control. Candle samples are labeled CN-A to CN-F. Error bars represent mean ±SD of triplicate determinations.

The results in Figures 3-11 and 3-12 show that the effect of the extracts from candle PM on the most sensitive indicators of PM exposure, CYP1A1 and COX-2, respectively, was relatively small. A detectable increase of about 2-fold above control levels was observed for CYP1A1 in U937 macrophages after exposure to extract from candles CN-B through CN-H. The expression of CYP1A1 in NCI H441 lung cells was not significantly changed by exposure to PM extract from the candles tested. The second parameter tested was the inflammatory enzyme COX-2. The highest increase of COX-2 of about 2-fold was found after treatment with CN-D followed by CN-C and CN-B in human U937 macrophages. Treatment with PM extract of BA increased COX-2 in NCI H441 lung cells about 2-fold, whereas other candle PM extracts did not significantly increase COX-2 in NCI H441 lung cells above the level of a blank filter.

In summary, the effects of PM candle extracts were somewhat weak compared to responses seen in the other complex PM sources tested. The response in CYP1A1 would indicate the possible presence of PAHs capable of interacting with AhR in the cell. Chemical analyses of PAHs in the candle samples will be discussed in a subsequent section of this report. Studies with higher concentrations of PM from candle burning would be needed to estimate the level necessary to generate significant effects on toxicity markers or inflammatory parameters. No significant change was observed on the expression of HO-1 in either cell line, indicating that exposure to extracts from candle PM did not generate a significant amount of oxidative stress capable of inducing HO-1 (data not shown).

The PM mass data are summarized in Table 3-8. PM mass varied widely, from about 0.15 to 2.6 mg per filter. The type of candle, whether container- or pillar-type, did not seem to correlate with the PM loading. However, the variability in level of PM may have been dependent on the PM plume characteristics and path to the inlet of the size-selective device.

3.3.3.3 Results: Continued Testing of Candles in Macrophages

We tested the effect of PM extracts from eight different candles. In this screening test we found that candle PM samples tested had only a slight effect on the most sensitive parameter CYP1A1 in both macrophages and NCI H441 lung cells. Since the activities did not seem to span a large range in any of the markers, and because candle PM did elicit activity (for example, IL-8 in macrophage cells) during development of the testing procedure, we wanted to confirm the results of some of the candles tested above. We therefore retested two candle samples.

Table 3-9 Continued Testing of Candle PM.

Sample	ID	PM2.5 Mass (mg/filter)	Adsorbent for Vapor Phase
Candle Scented Pillar Type	**CN-A**	**0.052**	**X**
Candle Scented Container Type	**CN-B**	**1.4750**	**X**
Background Air	**BGA**	**0.001**	**X**

The samples were PM from candles CN-A and CN-B. We tested these two candle samples only in the sensitive human U937 macrophages at a standard concentration of 10 μg/ml which was used for the candle samples tested before.

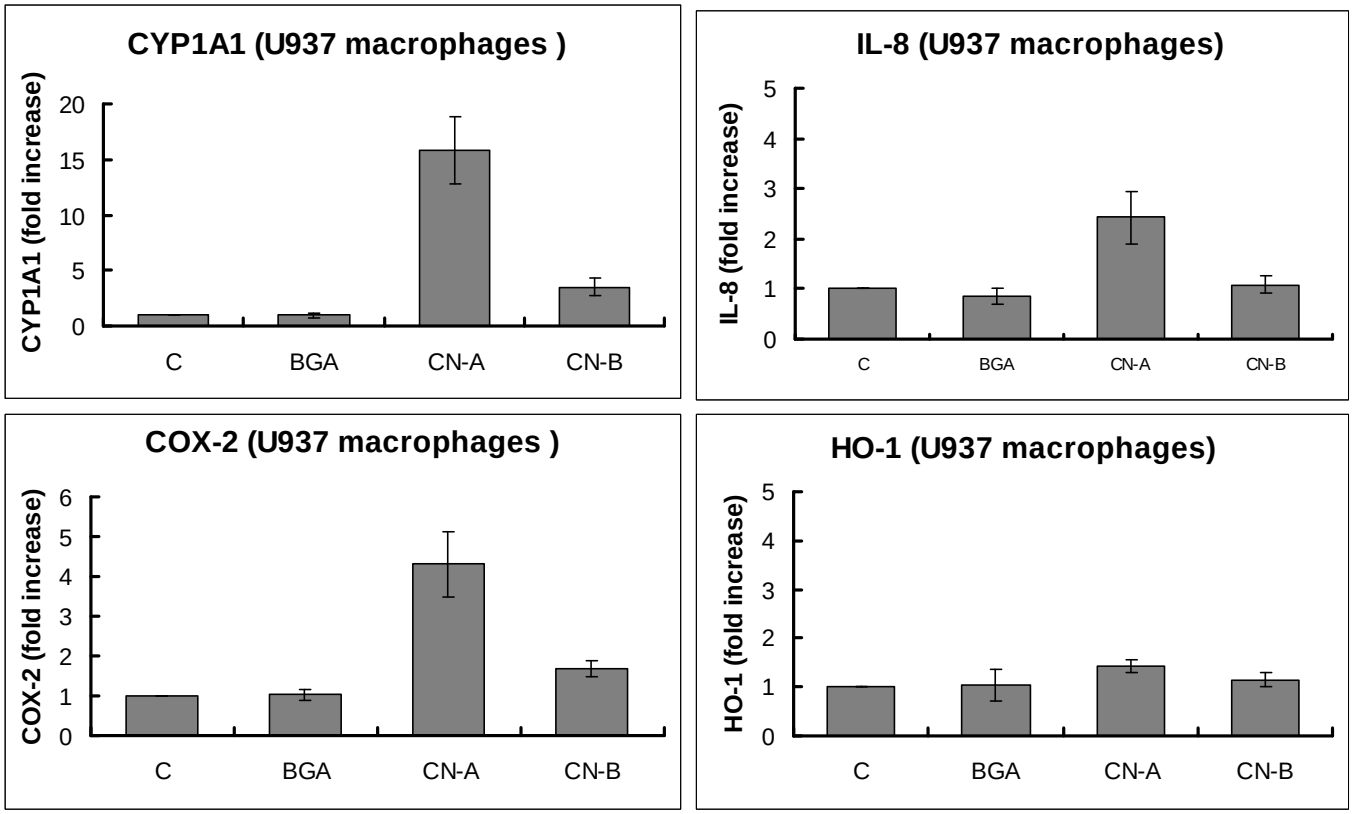


Figure 3-13. Effect of candle indoor source PM samples on CYP1A1, COX-2, IL-8, and HO-1 mRNA expression in U937 macrophages. Cells were treated for 24 hr with 10 µg/ml PM equivalent organic extract. Vehicle control (C) and background air (BGA) were included as controls. Candle samples are labeled candle CN-A and CN-B. Error bars represent mean ± SD of triplicate determinations.

Unexpectedly, the expression of CYP1A1 mRNA as illustrated in Figure 3-13 was significantly induced by about 16-fold above background air (BA) after treatment with PM extract from the candle CN-A. The PM sample from sample CN-B had only a slight effect (3-fold) on the expression of CYP1A1. The inflammatory markers COX-2 and IL-8 were analyzed in U937 macrophages and were only induced by CN-B PM extract by about 4 and 2.5-fold, respectively. Treatment with the PM sample extract from CN-B did not significantly change the expression of COX-2 or IL-8 in U937 macrophages

The expression of the oxidative stress marker HO-1 was not significantly changed by candle PM extracts from candle CN-A or candle CN-B compared to the background air sample (BGA).

To determine the amount of candle mass burned during the sampling period, we tested the two candles A and B as is summarized in Table 3-10. The candles represent the pillar type and the container type. Both types have similar burn rates. Fan and Zhang (2001) reported on the emissions of some candles in

a small desktop size chamber. They reported a burn rate for four 3" candles to be 15.2 g per hour or about 3.8 g per candle per hour. This is approximately the amount of candle burned in our test (Table 3-7). Fan and Zhang (2001) also reported that the particle diameters were predominantly less than 1 µm especially when extinguishing the flame. Fine et al. (1999) also reported that candles (as collected in an enclosed chamber) had particle mass diameters less than 1 µm.

Table 3-10. Candle mass burned and burn rate.

Sample	ID	Time (min)	Total Wt. Candle Burned (g)	Material Burn Rate (g/min)
Candle Scented Pillar Type	**CN-A**	**60**	**3.48**	**0.058**
Candle Scented Container Type	**CN-B**	**60**	**3.61**	**0.060**

3.3.3.4 Summary

In summary, the results show that PM from some candles (for example candle CN-B) may contain a considerable amount of PAHs, which can lead to the induction of the AhR-regulated gene CYP1A1 and inflammatory marker genes COX-2 and IL-8. However, many of the initial candle PM samples tested did not appear to induce inflammatory marker genes such as COX-2 and IL-8. However, the data also indicate that some specific candle products may contain toxic components, which are capable of generating toxicity in specific target cells.

Further investigation is needed to identify the source of the components and/or the characteristics of the PM from candle products which may exert significant signs of toxicity as found for candle sample CN-B.

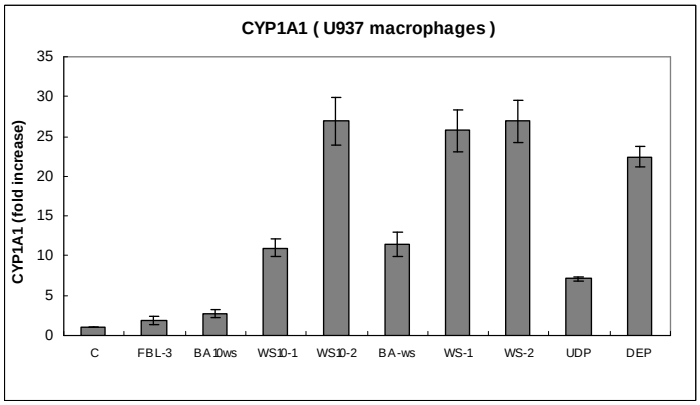
3.3.4 Woodsmoke PM

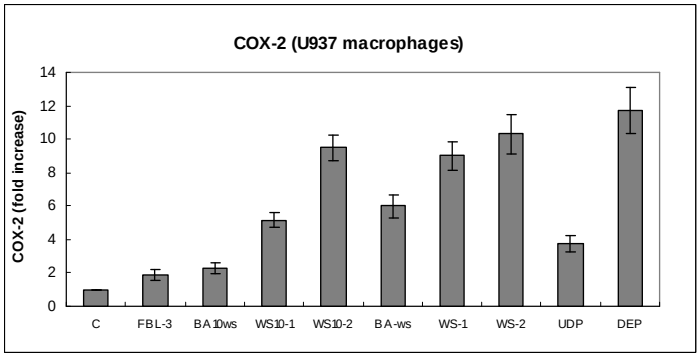
The PM mass levels per filter for the woodsmoke tests are presented in Table 3-11. The PM2.5 and PM10 samples for each trial were obtained in parallel. The adsorbent cartridge (PUF/XAD) was used for the chemical analyses phase (Section 4). Trial 1 contained less PM2.5 and PM10 than Trial 2, which reflects the increased level of visible smoke in the house during Trial 2. The PM masses for Trial 1 are lower than Trial 2 probably due to a higher intensity of fire in Trial 2. There was also noticeable smoke coming into the room.

Table 3-11 PM mass measurements of woodsmoke samples.

Sample	ID	PM2.5 Mass (µg/filter)	PM10 Mass (µg/filter)	Adsorbent for Vapor Phase
Woodsmoke Burn 1 (Trial 1)	**WS-1**	**38**	**74**	**X**
Woodsmoke Burn 2 (Trial 2)	**WS-2**	**114**	**154**	**X**
Background Air	**BGA**	**27**	**68**	**X**

Indoor PM samples from woodsmoke (IDs: WS10 or WS for PM10 and PM2.5 samples, respectively) were tested in human macrophages U937. Cell lines were treated for 24 hr with 10 µg/ml PM-equivalent of the organic extract. As positive controls, cells were treated with 10 µg/ml urban dust particles (UDP, NIST SRM 1649) extract and 10 µg/cm^2 diesel engine exhaust (DEP, SRM 2957) extract. The mRNA expression of CYP1A1, COX-2, and IL-8 were analyzed using real-time PCR.





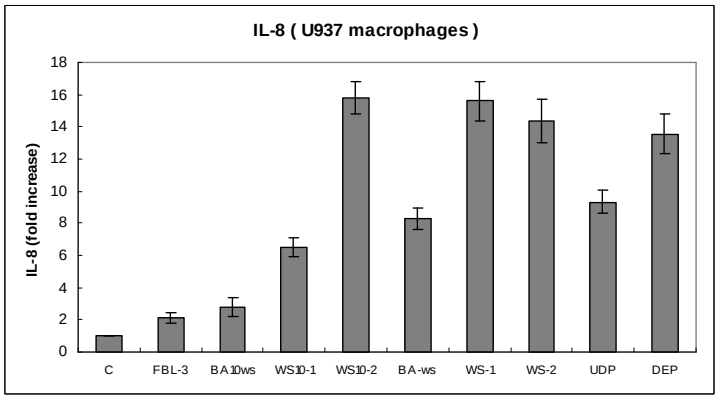
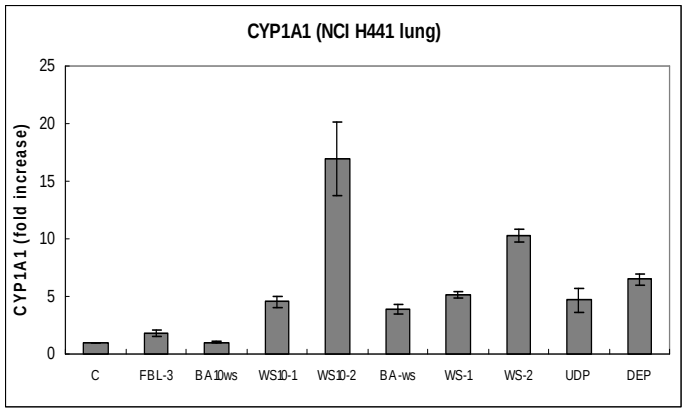
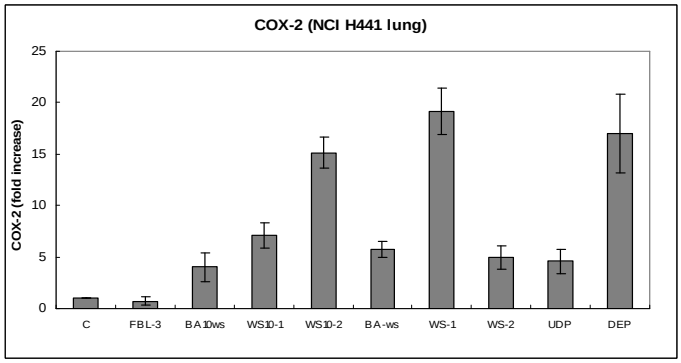


Figure 3-14. Effect of extracts from woodsmoke source PM samples on cytochrome P450 1A1 (CYP1A1), COX-2, and IL-8 mRNA expression in U937 macrophages.
Cells were treated for 24 hr with 10 µg/ml (PM equivalent) organic extract from woodsmoke source PM samples. As a positive control cells were treated with 10 µg/ml urban dust (UDP) or 10 µg/ml diesel engine emissions extract (DEP). Error bars represent mean ± SD of triplicate determinations.





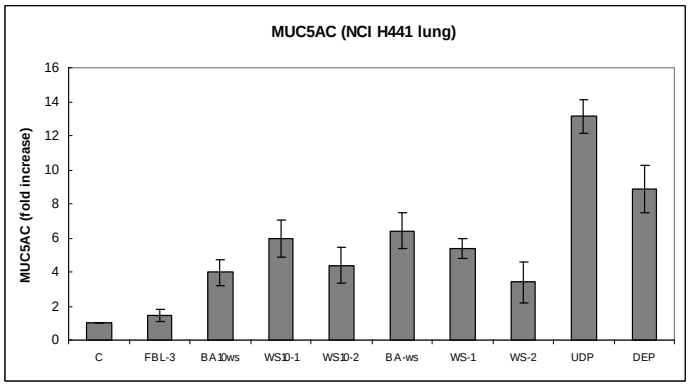


Figure 3-15. Effect of extracts from woodsmoke source PM samples on cytochrome P450 1A1 (CYP1A1), COX-2, and MUC5AC mRNA expression in NCI H441 human cell lines.
Cells were treated for 24 hr with 10 µg/ml (PM equivalent) organic extract from woodsmoke source PM samples. As a positive control cells were treated with 10 µg/ml urban dust (UDP) or 10 µg/ml diesel engine emissions extract (DEP). Error bars represent mean ± SD of triplicate determinations.

LEGEND

FBL	Filter Blank	BA-ws	Background Air PM 2.5
BA10	Background Air PM10	WS-1	Woodsmoke PM2.5 run#1
WS10-1	Woodsmoke PM10 run # 1	WS-2	Woodsmoke PM2.5 run#2
WS10-2	Woodsmoke PM10 run # 2	UDP	Urban Dust NIST SRM 1649
DEP	Diesel PM NIST SRM2975		

Figure 3-15 illustrates cells treated with the indoor PM source samples and the response of the human macrophages U937 for CYP1A1, COX-2, and IL-8, respectively. Samples WS10-2 and WS-1 and WS-2 (PM10 woodsmoke second trial; PM2.5 for WS-1 and WS-2) had the highest response regarding the induction of CYP1A1. The responses for CYP1A1 were higher than observed with the positive controls UDP, or DEP at 10 µg/ml PM equivalents (Figure 3-14, top) in U937 macrophages.

In parallel experiments we treated and tested the NCI H441 human lung cell line (NCI H441) for the three biological markers of PM toxicity, the xenobiotic metabolizing enzyme cytochrome P4501A1 (CYP1A1), the inflammatory enzyme cyclooxygenase 2 (COX-2) and mucin producing gene MUC5AC. The cells were treated for 24 hr with 10 µg/ml PM equivalent of the organic extract. As positive controls, cells were treated with 10 µg/ml UDP and 10 µg/ml DEP. Figure 3-15 illustrates cells treated with woodsmoke source samples and the response of the human NCI H441 human lung cell line for CYP1A1 and COX-2, respectively. Samples WS10-2, WS-1, WS-2 (woodsmoke PM10 and PM2.5) had the highest response regarding the induction of CYP1A1. Similar results were obtained for the inflammatory marker gene COX-2 (Figure 3-15) with the exception that the positive controls treated with DEP extract had the highest level for COX-2 followed by woodsmoke samples WS10-2, WS-1, and WS-2.

The most significant effect on CYP1A1 and COX-2 expression in the human NCI H441 cells was found for the extract #2 from woodsmoke PM10 and PM2.5. As in macrophages, the effects of the woodsmoke extracts were similar or even higher than those generated after treatment with positive controls of UDP and DEP.

The results further showed no significant increase in the level of the secretory protein mucin 5 (MUC5AC) after treatment with woodsmoke PM compared to background air PM. Only the positive control extracts from UDP and DEP had significant effects on MUC5AC expression in NCI H441 lung cells (Fig 3-15, bottom) indicating that the toxicity from woodsmoke PM might be relatively small regarding the over-expression of mucin. However, the woodsmoke PM tested in this study had similar or even greater effects on the induction of CYP1A1 and inflammatory marker genes like COX-2 and IL-8, suggesting the toxic potency of woodsmoke PM.

3.3.4.1 Summary

The most significant effect on CYP1A1 and COX-2 expression in the human NCI H441 cells was found for the extract #2 from woodsmoke PM10 and PM2.5. In general the effects of PM10 and PM2.5 collected from woodsmoke were comparable except for some cases like COX-2 induction in NCI H441 cells where the effect of PM2.5 was slightly higher than PM10. As in macrophages, the

effects of the woodsmoke extracts were similar or even higher than those generated after treatment with positive controls of UDP and DEP.

The results further showed no significant increase in the secretory protein mucin 5 (MUC5AC) after treatment with woodsmoke PM compared to background air PM. Only the positive control extracts from UDP and DEP had significant effects on MUC5AC expression in NCI H441 lung cells (Figure 3-15), indicating that the toxicity from woodsmoke PM might be relatively small regarding the over-expression of mucin. However, the woodsmoke PM tested in this study had similar or even greater effects on the induction of CYP1A1 and inflammatory marker genes like COX-2 and IL-8 suggesting the toxic potency of woodsmoke PM.

3.3.5 Incense PM

3.3.5.1 Effects of Incense PM on various marker genes.

Incense burning is an important rite in daily religious ceremonies for a great percentage of families in some parts of Asia, and is getting to be a more common and popular practice in many households of the Western world. Churchgoers and temple workers are potentially exposed to high concentrations of various pollutants emitted from incense burning. Chronic cough and development of acute irritation symptoms, including nose and throat irritation, have been reported.

Incense burning generates a large amount of particulate and gaseous pollutants (Kao & Lung, 2000; Fang et al., 2002; Lung & Hu, 2003). Due to the nature of its slow and incomplete combustion, this practice produces a continuous stream of smoke. The smoke emitted by incense burning has been reported to contain PAHs (Kao & Lung, 2000; Lung & Hu, 2003) and aliphatic aldehydes (mainly formaldehyde) (Lin & Wang, 1994; Lin & Tang, 1994) and has also been found to be mutagenic in the Ames *Salmonella* test (Sato, et al.,1980; Rasmussen, 1987). The generated pollutants are easily accumulated indoors, especially under inadequate ventilation. It is therefore possible that people practicing indoor incense burning are exposed to high levels of PAHs, formaldehyde, and PM originating from incense burning.

Particle mass and number measurements in a church resulted in significant increases of indoor particle concentrations during the burning of incense. Generally, varying concentration regimes can be attributed to different "modes of indoor activity" and emission sources. While periods of candle burning produced negligible concerning particle concentrations, significant increases (7-fold) in PM10 and PM2.5 concentrations have been measured during incense burning in churches (Weber, 2006). There have been reports that PM10 levels reached an approximate 8-fold increase in comparison to outdoor measurements (Weber, 2006). The increase of particles < 2 µm was significantly enhanced in comparison to larger particles and concentrations were still elevated above indoor background concentrations for approximately 24 hr.

3.3.5.2 Results: Incense PM

During the pilot study it was demonstrated that incense PM samples had significant and dose-dependent effects on CYP1A1 as well as other inflammatory and oxidative stress markers in both macrophages and NCI H441 lung cells. In order to test whether incense samples from different sources and manufacturers have a different effect on the selected marker genes, we decided to include six

additional PM samples from various incense sources. The incense samples tested were: Incense, Nag Champa (INC-A); Incense, Pure Tibetan (INC-B); Incense Shoyiedo Japanese (INC-C); Incense, Potala (INC-D); Incense, Aromatherapy variety (INC-E). Incense, Joss Stick green (INC-F); and Incense, Floral Variety (INC-G).

Table 3-12 PM mass measurements of incense samples.

Sample	ID	PM 2.5 Mass (mg/filter)
Incense Nag Champa	INC-A	7.82
Incense Tibet	INC-B	9.97
Incense Shoyiedo	INC-C	15.0
Incense Potala	INC-D	2.65
Incense Aromatherapy	INC-E	4.16
Joss Stick green	INC-F(a)	6.24
Joss Stick green	INC-F(b)	1.38
Floral Variety	INC-G	7.24
Floral Variety	$INC\text{-}G_{10}$	9.51[a]
Background air	BGA	.04

[a]PM10

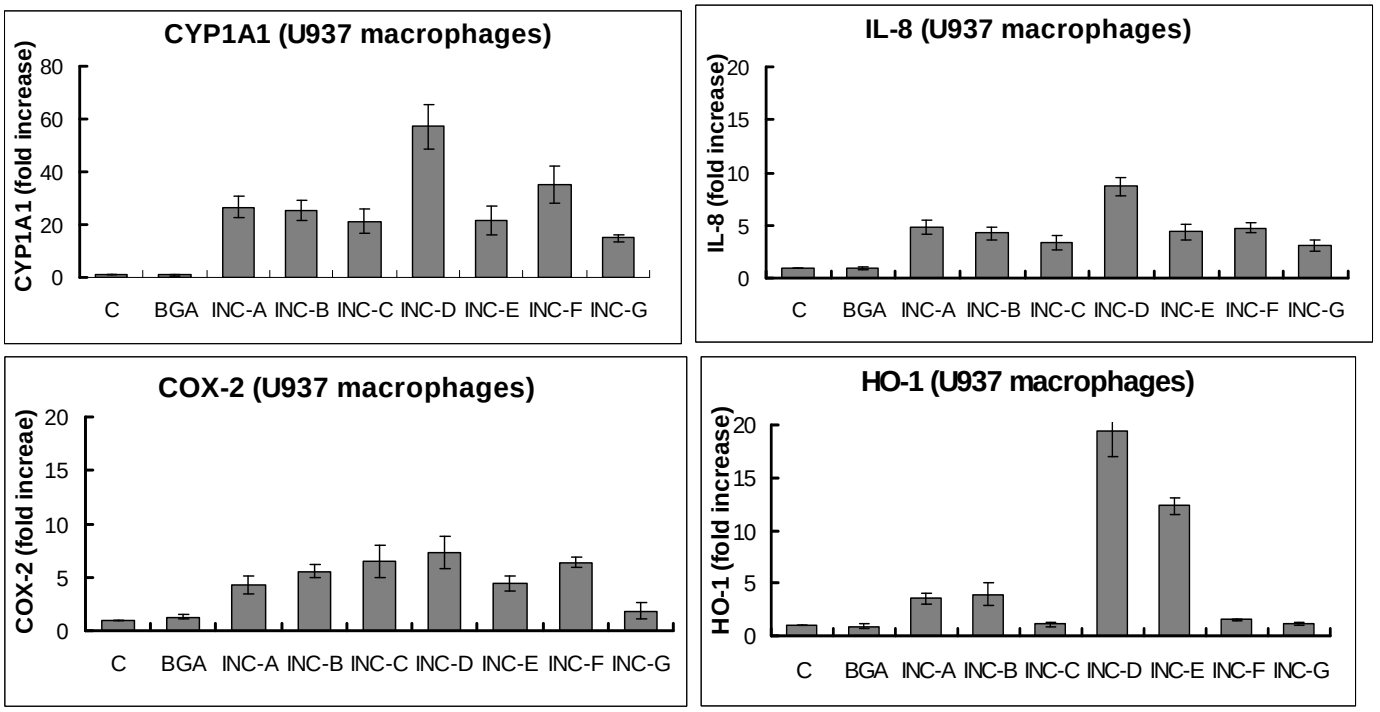


Figure 3- 16. Effect indoor source incense PM samples on CYP1A1, IL-8, COX-2, and HO-1 mRNA expression in U937 macrophages.

Cells were treated for 24 hr with 2.5 µg/ml PM equivalent organic extract. Vehicle control DMSO (C) and background air (BGA) were used as control. Incense samples are labeled INC-A to INC-E. Error bars represent mean ± SD of triplicate determinations.

In an effort to conserve samples, we initially chose to test our panel of incense samples in only sensitive human U937 macrophages at a medium dose of 2.5 µg/ml. The expression of CYP1A1 mRNA was significantly induced by all seven incense PM samples tested. The highest increase of about 60-fold was observed after treatment with Incense, Potala (INC-D). The other incense samples tested increased CYP1A1 by about 30-fold compared to background air samples. Similar results were obtained when the inflammatory markers COX-2 and IL-8 were analyzed in U937 macrophages. The highest potency was found for Incense, Potala (INC-D) followed by the remaining incense samples, which were relatively similar.

Analysis of the oxidative stress marker HO-1 indicated a strong effect of about 20- and 12-fold for Incense, Potala (INC-D) and Incense, Aromatherapy variety (INC-E), respectively. Interestingly, the effect of Incense, Nag Champa (INC-A), and Incense, Pure Tibetan (INC-B) were considerably weaker on HO-1 expression compared to the other incense samples (INC-E and INC-F) tested. Incense Shoyiedo Japanese (INC-C) had no significant effect on the expression of HO-1 compared to the background air sample (INC-D). First, these results confirm that the induction of the inflammatory marker COX-2 and IL-8 are triggered by different signaling pathways, and second, the data suggest that the various incense samples tested contain different components or a different amount of those components, which can induce oxidative stress but not inflammatory markers. This is important, since the generation of oxidative stress can be associated with a genotoxic and mutagenic potential of the incense samples.

The amount of incense mass burned during the sampling period is summarized in Table 3-13. The incense sticks tested (INC-G) had very similar mass lost and the burn rates were therefore very similar.

Table 3- 13 The amount of incense mass burned during the sampling period.

Sample	ID	Time (min)	Mass Incense Burned (g)	Material Burn Rate (g/min)
Incense – stick 1	**INC-G**	**45**	**1.1859**	**0.026**
Incense – stick 2	**INC-G**	**45**	**1.2686**	**0.028**
Incense – stick 3	**INC-G**	**45**	**1.3222**	**0.029**
Incense – stick 4	**INC-G**	**45**	**1.1432**	**0.025**
			Ave	**0.027**
			SD	**0.002**

3.3.5.3 Results: Testing of Incense PM dose response

The purpose of this study was to assess whether exposure to PM from incense may result in a dose-dependent increase of inflammatory markers. These increases may reflect potential health effects, resulting in chronic or acute respiratory symptoms in people using incense in their household. We continued to investigate the dose-response relationships of incense samples as an indoor source PM on their effects on the macrophage cell line U937 and the human NCI H441 lung cells.

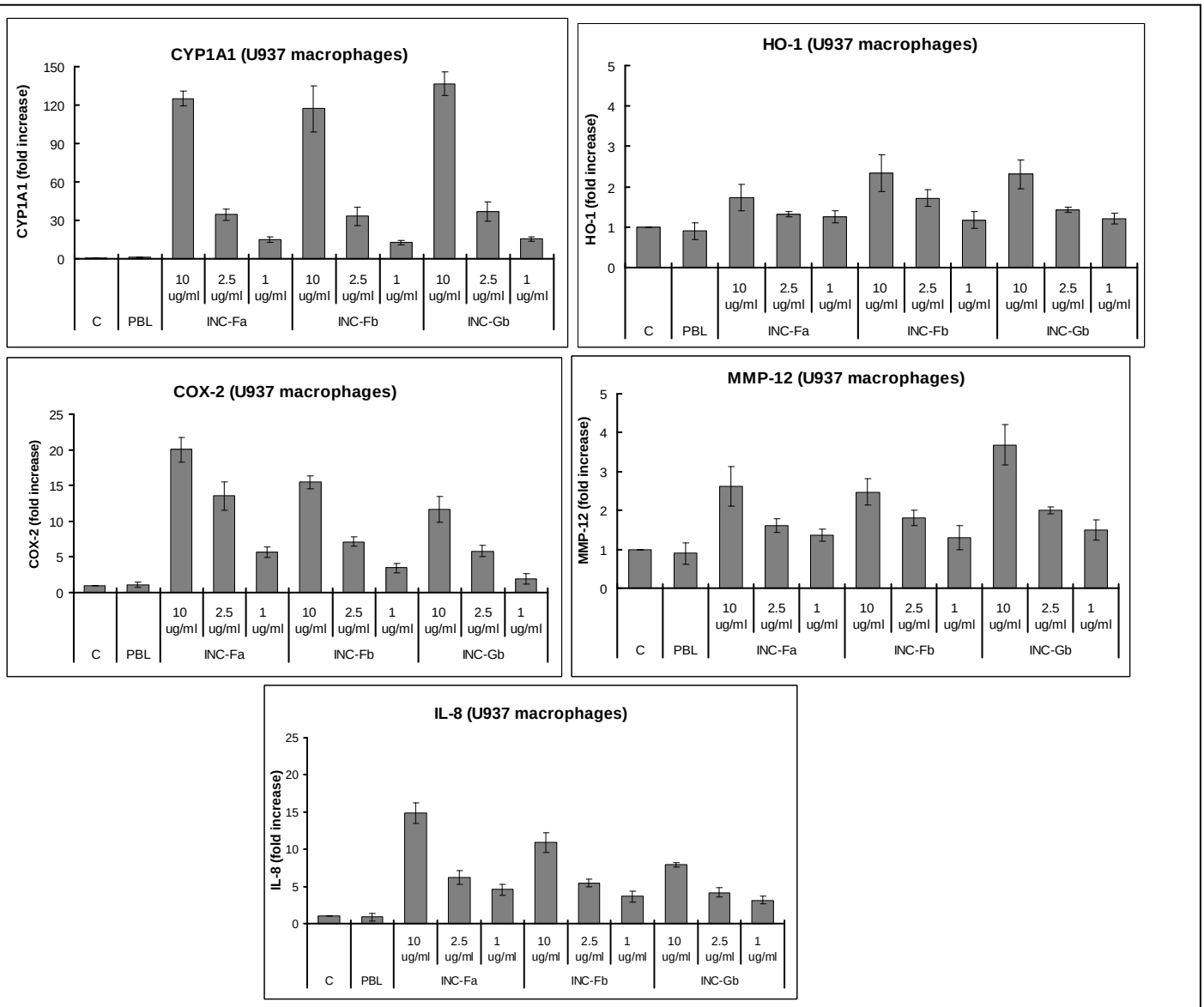


Figure 3-17. **Dose response relationships from three indoor-source incense PM samples on cytochrome P4501A1 (CYP1A1), HO-1, COX-2, MMP-12, and IL-8 mRNA expression in U937 macrophages.**
Cells were treated for 24 hr with concentrations of 1, 2.5, and 10 µg/ml particle equivalent organic extracts from incense source PM samples. Vehicle control DMSO (C) and process blank (PBL) are the first bars on the left. Incense samples are labeled INC-Fa, INC-Fb and INC-Gb. Error bars represent mean ± SD of triplicate determinations.

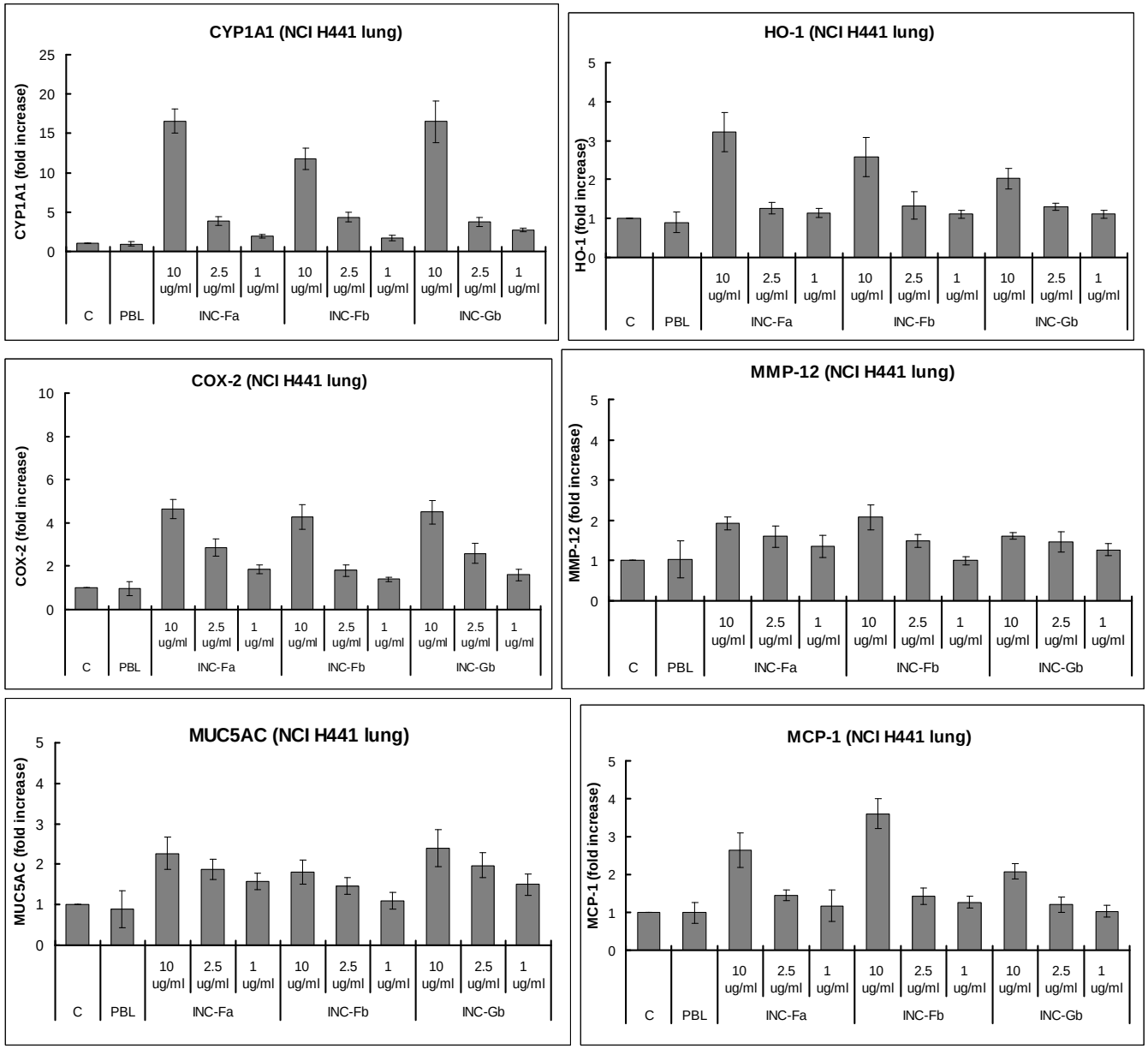


Figure 3-18. Dose response relationships from three indoor-source incense PM samples on cytochrome P4501A1 (CYP1A1), HO-1, COX-2, MMP-12, and MCP-1 mRNA expression in NCI-H441 cells .
Cells were treated for 24 hr with concentrations of 1, 2.5, and 10 µg/ml particle equivalent organic extracts from incense source PM samples. Vehicle control DMSO (C) and process blank (PBL) are the first bars on the left. Incense samples are labeled INC-Fa, INC-Fb and INC-Gb. Error bars represent mean ± SD of triplicate determinations.

All three incense samples (INC-Fa, INC-Fb, and INC-G) were very active in increasing expression of CYP1A1, IL-8 and COX-2 induction (Figure 3-17). The expression of HO-1 (an indicator for oxidative stress) and MMP-12 (an atherogenic marker for cholesterol-accumulating macrophages) was significantly increased by all three incense samples but only at the highest concentration of 10 μg/ml. It is noteworthy that induction of HO-1 has not been observed at the same concentration of 10 μg/ml with UDP, DEP or the positive control of AhR activation with TCDD. The expression levels of CYP1A1, COX-2, and IL-8 in macrophages were increased by all three incense samples tested in a dose-dependent manner (Figure 3-17). The results show that there is expression of the inflammatory marker genes.

We continued to investigate the effect of incense samples on their effect on the NCI H441 lung Clara cell line (Figure 3-18). Incense from a various suppliers was investigated. Three different PM extracts of incense were tested and found to be very active in increasing expressions of CYP1A1, IL-8 and COX-2 induction in U937 macrophages. Here we tested the effect of the same incense extracts in NCI H441 human cells and further investigated the dose-response relationship of the incense samples used for the macrophage assay. The NCI H441 cell line is derived from a human bronchiolar Clara cell which is a non-ciliated epithelial cell line present as a major cell type on the surface of small (< 2 mm) airways. Further testing of this cell line involved testing a second set of incense samples. Markers for CYP1A1, COX-2, and MUC5AC were increased in a dose-dependent manner (data not shown). A detectable increase of all three marker genes was found at the lowest concentration (1 μg/ml) tested.

In view of the exceptionally high increase of inflammatory markers elicited by relatively low concentrations of incense PM (1 μg/ml) and the high level of AhR potency and the presence of PAHs (see chemical analysis), combined with a high radical-generating capacity indicated by significant increase of HO-1 expression, regular exposure to incense-derived PM is likely to result in increased risk of pulmonary or cardiovascular effects.

3.3.6 Results summary - Comparison of PM sample groups

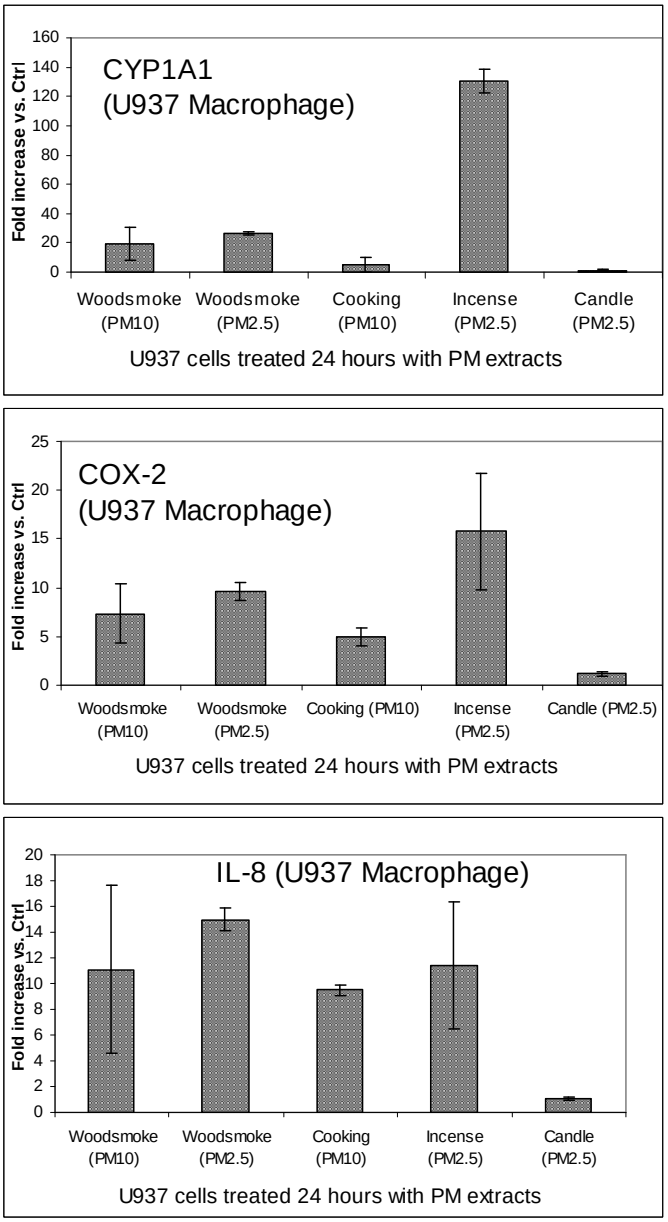


Fig 3-19 Expression of CYP1A1, COX-2 and IL-8 in U937 cells for all indoor source PM samples tested.

Cells were treated for 24 hr with concentrations of 10 µg/ml particle equivalent organic extracts from indoor source PM samples. Error bars represent standard deviation of the responses between different types of the same indoor source.

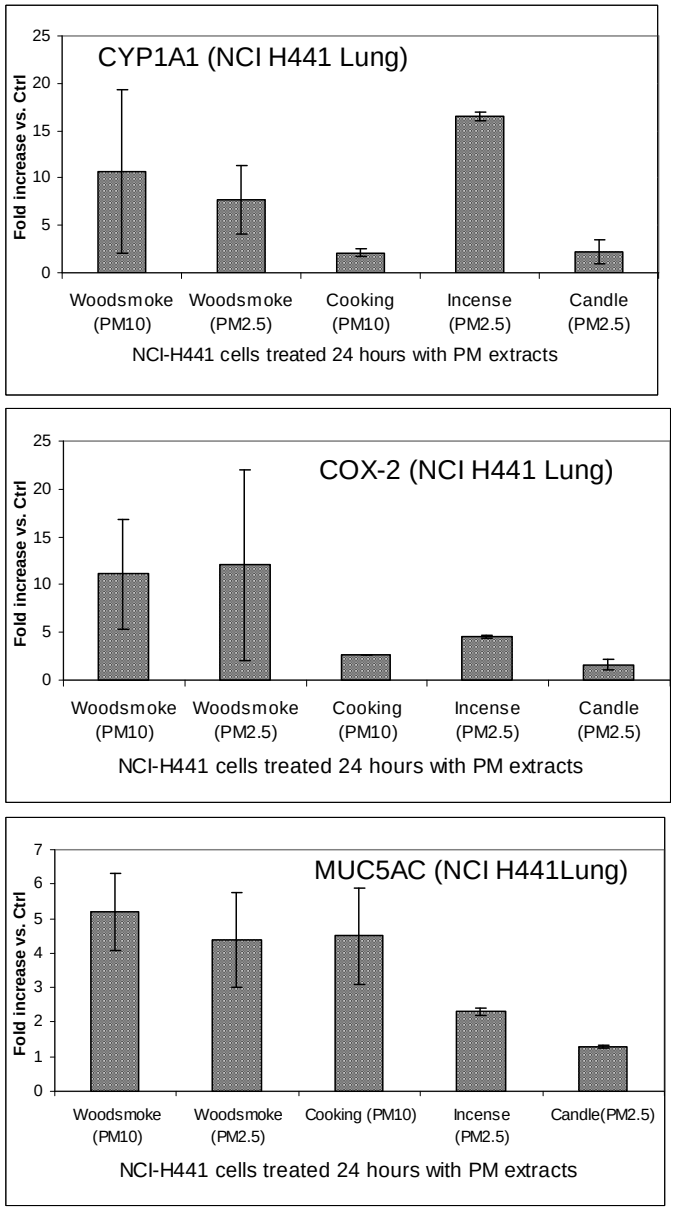


Figure 3-20 Expression of CYP1A1, COX-2 and MUC5AC in NCI-H441 cells for all indoor source PM samples tested.

Cells were treated for 24 hr with concentrations of 10 µg/ml particle equivalent organic extracts from indoor source PM samples. Error bars represent standard deviation of the responses between different types of the same indoor source.

Figures 3-19 and 3-20 represent comparative results for different treatments of indoor pollutants with either U937 macrophages (Figure 3-18) and NCI H441 lung cells (Figure 3-20). Analysis within and between the two figures illustrated:

1) In general, U937 is more sensitive than NCI H441 cells line for measuring molecular markers of inflammation and oxidative stress for the indoor source PM samples tested.
2) Woodsmoke has the widest variability in its results probably due to difficulties in controlling burn rates
3) There does not appear to be much difference in particle size for the biological effects due to woodsmoke
4) Incense elicits the highest response in both human cell lines especially for CYP1A1.

4.0 BIOLOGICAL AND CHEMICAL CHARACTERIZATION OF PM

Objective: To incorporate and evaluate the use of bioassay-directed chemical characterization of the most toxic of the indoor source PM samples, and initially chemically characterize the PM. (Main study-goal 3)

4.1 Introduction

To broadly characterize the most potent indoor PM source, incense PM, we incorporated the approach of using bioassay to tell us which possible chemical fraction (polar, non-polar) the active components were located. We then wanted to generally identify general chemical classes that may be present in these fractions. This information may provide some idea of which components are possibly active in the bioassay.

First, the organic extracts of the most potent PM sample were fractionated by polarity, and bioassay was conducted for each fraction to biologically and chemically characterize the PM. The extract from incense PM had the highest potency of the indoor PM sources tested in the bioassay analyses. The purpose of the chemical fractionation was to help characterize the components with the highest potency to induce toxicity, inflammatory or oxidative stress marker. We therefore tested polar, semi-polar and non-polar fractions of three different sources of incense PM. Second, the incense PM sample was further investigated for the mechanism of action that induces CYP1A1.

For chemical characterization of indoor PM, first our investigation focused on polycyclic aromatic hydrocarbons (PAHs) in the PM. PAHs are generated by incomplete combustion and the indoor PM sources we investigated all involved heating reactions of carbonaceous compounds. Also, some PAHs are known carcinogens and can induce CYP1A1 by activating Aryl hydrocarbon-Receptor (AhR) (Santodonato et al., 1983). Therefore, PAHs are important to study in the indoor PM samples by chemical analyses in conjunction with biological analyses. PAHs were investigated initially by real-time monitoring and also by quantitative chemical analysis. Second, presence of other compounds were characterized by gas chromatography/mass spectrometry (GC/MS) for indoor PM samples from candle, cooking, woodsmoke, and incense. Their relevance to the biological response is discussed.

4.2 Bioassay-Directed PM fractionation

The extract from incense PM had the highest potency of the indoor PM sources tested in the bioassay analyses. To biologically and chemically characterize the incense PM, a chemical fractionation method was used in conjunction with the bioassay. The purpose of the chemical fractionation was to determine which chemical fraction had the highest potency to induce toxicity, inflammatory or oxidative stress markers. We therefore tested polar and non-polar fractions of three different sources of incense PM.

The chemical fractionation of a complex PM emission presents challenges due to the number of compounds present. This could be accomplished through liquid-liquid extraction procedures or by chromatography. We chose the latter by using silica as adsorbent since in our laboratory we have run a number of complex mixtures from combustion PM through this procedure to isolate PAHs and nitro-PAHs. Briefly, a fractionation procedure starting with non-polar solvents moving toward more polar solvents was used. The first fraction was eluted with hexane, followed by DCM/hexane mixture (2:9, v/v) as the second fraction, followed by DCM/hexane mixture (1:1, v/v) as the third fraction, followed by methanol as the forth fraction. Fractionation profile of PAH standard chemicals ranging in size from 2-rings (naphthalene) to 6-rings (benzo[ghi]perylene) and nitro-PAH standards from 2-rings (1-nitronaphthalene) to 6-rings (6-nitrobenzo[a]pyrene) was investigated and illustrated in Figure 4-1. Fraction 1 (F1) was the most non-polar, and fraction 2 (F2) with slight polarity contained PAHs and some nitro-PAHs. Fraction 3 (F3) contained more polar compounds including the nitro-PAHs. Fraction 4 was the most polar.

For the fractionation experiment on the incense samples, the incense extracts in DCM were solvent exchanged into hexane. The hexane extract was added to a mini-column of pre-cleaned silica that was baked at 550 ℃ for 8 hr prior to use to eliminate possible organic contaminants. The silica column was eluted with solvents with different polarities as described above, and the fractions could then be tested by bioassay.

For the biological analyses of the incense extract fractions, we analyzed CYP1A1, COX-2 and HO-1 expression in U937 macrophages and NCI H441 lung cells after treatment with 10 μg/ml PM equivalent extract from incense samples (INC-F, INC-Ga, and INC-Gb) for 24 hr. We tested the total extract or un-fractionated “neat” extract of each incense sample and their corresponding fractions F1 to F4. The results are presented in Figures 4-2 to 4-4.

For CYP1A1 in both cell lines, U937 macrophages and NCI H441 cells, the same trend was observed for all the three incense extracts tested. The highest increase of CYP1A1 after treatment with the raw or neat (un-fractionated)

material was found, followed by the most polar fraction 4 (F4). The effect of F3 and F2 was also significant but lower than the effect of F4 at the same concentration. Treatment with the most non-polar fraction F1 had no significant effect on the expression of CYP1A1 in both cell lines tested. The same trend was observed for COX-2 in U937 macrophages. For HO-1 in macrophage cells, again F4 elicited the highest induction of the fractions. Fractions F1, F2, and F3 evoked similar levels. In NCI H441 cells, both COX-2 and HO-1 were higher in the neat extract than the corresponding fractions, but the difference among the fractions was small. The results show the high increase of CYP1A1 in macrophage and Clara cells is largely attributed to components contained in the most polar chemical fraction of the incense, although semi-polar fractions contribute to the toxicity also. This indicates that there may be important classes of compounds present in the incense PM that induce CYP1A1 that are more polar than PAHs or nitro-PAHs.

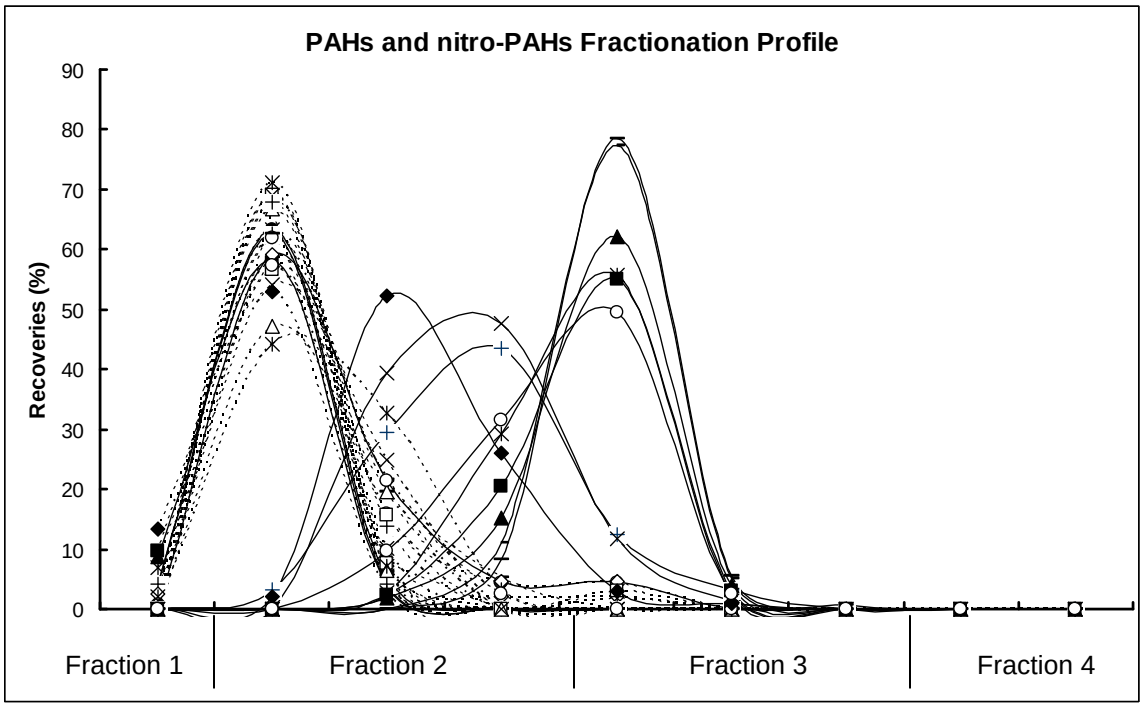


Figure 4-1. Chemical fraction of PAHs (2-ring to 6 ring) and nitro-PAHs (2-ring to 5-ring) standards illustrating the fractions collected for the bioassay.

The x-axis represents the chemical fractions starting with the least polar and ending with the most polar.
The different dotted lines represent individual PAH standards (typically present in fraction 2 (hexane/DCM 9:2, v/v) and solid lines for individual nitro-PAHs present both in fraction 2 and fraction 3 (hexane/DCM 1:1, v/v). Fraction 4 was the most polar fraction, eluted with methanol.

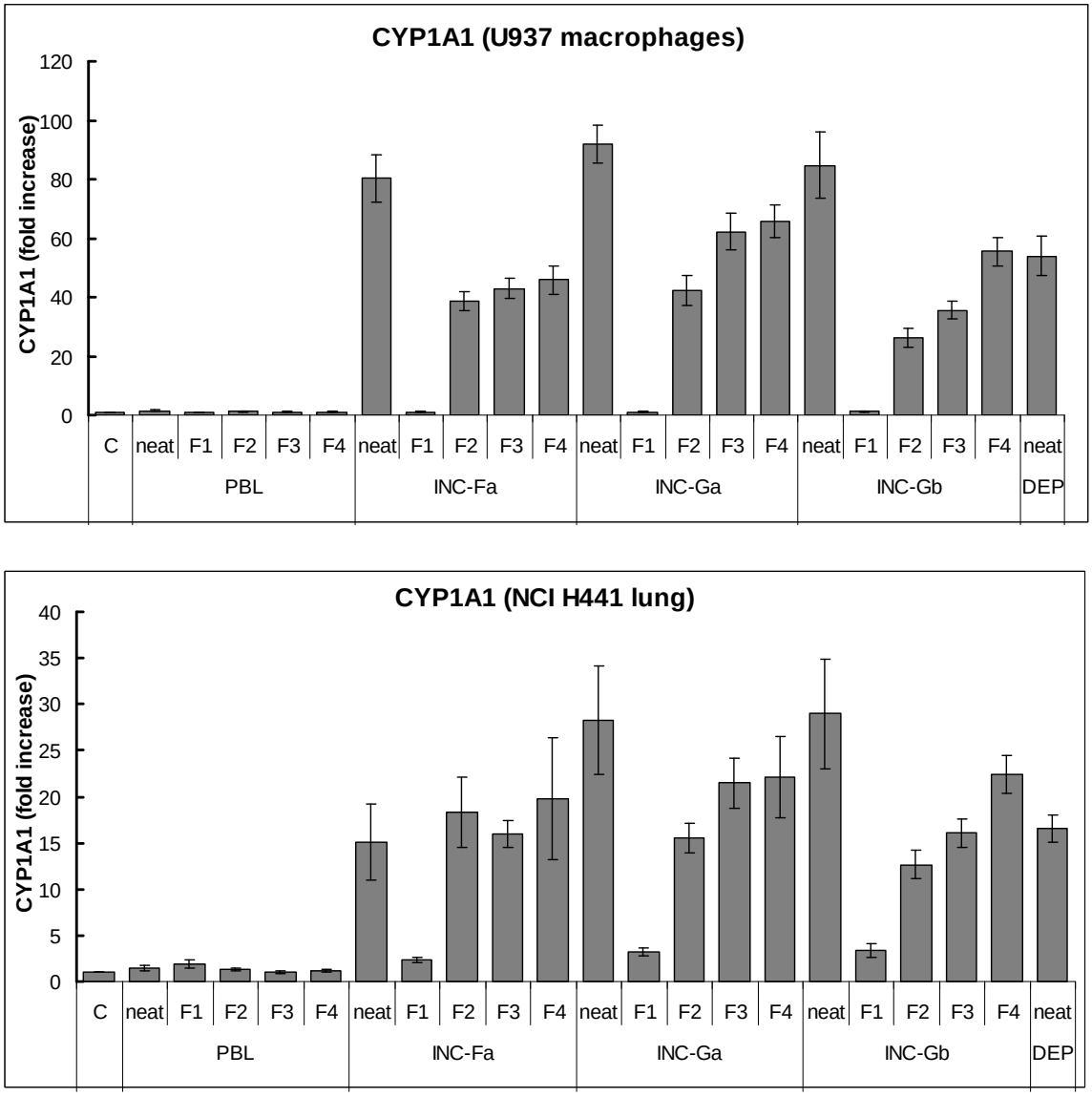


Figure 4-2. Effect of extracts from three indoor source incense PM samples on cytochrome P4501A1 (CYP1A1) mRNA expression in U937 macrophages and NCI H441 Clara lung cells.

Cells were treated for 24 hr with 10 µg/ml particle equivalent from "neat" un-fractionated extract or the fractions F1 to F4 from three different incense source PM samples. Error bars represent mean ± SD of triplicate determinations.

PM Sample ID:
C: Vehicle control
PBL: Process blank
INC-Fa: PM2.5 Incense, Mainichi-koh Japanese-1
INC-Ga: PM2.5 Incense, Floral India-1
INC-Gb: PM2.5 Incense, Floral India-2 duplicate
DEP: Diesel engine exhaust particles (NIST 2975)
F1= hexane; F2= hexane/DCM (9:2); F3= hexane/DCM (1:1); F4= methanol

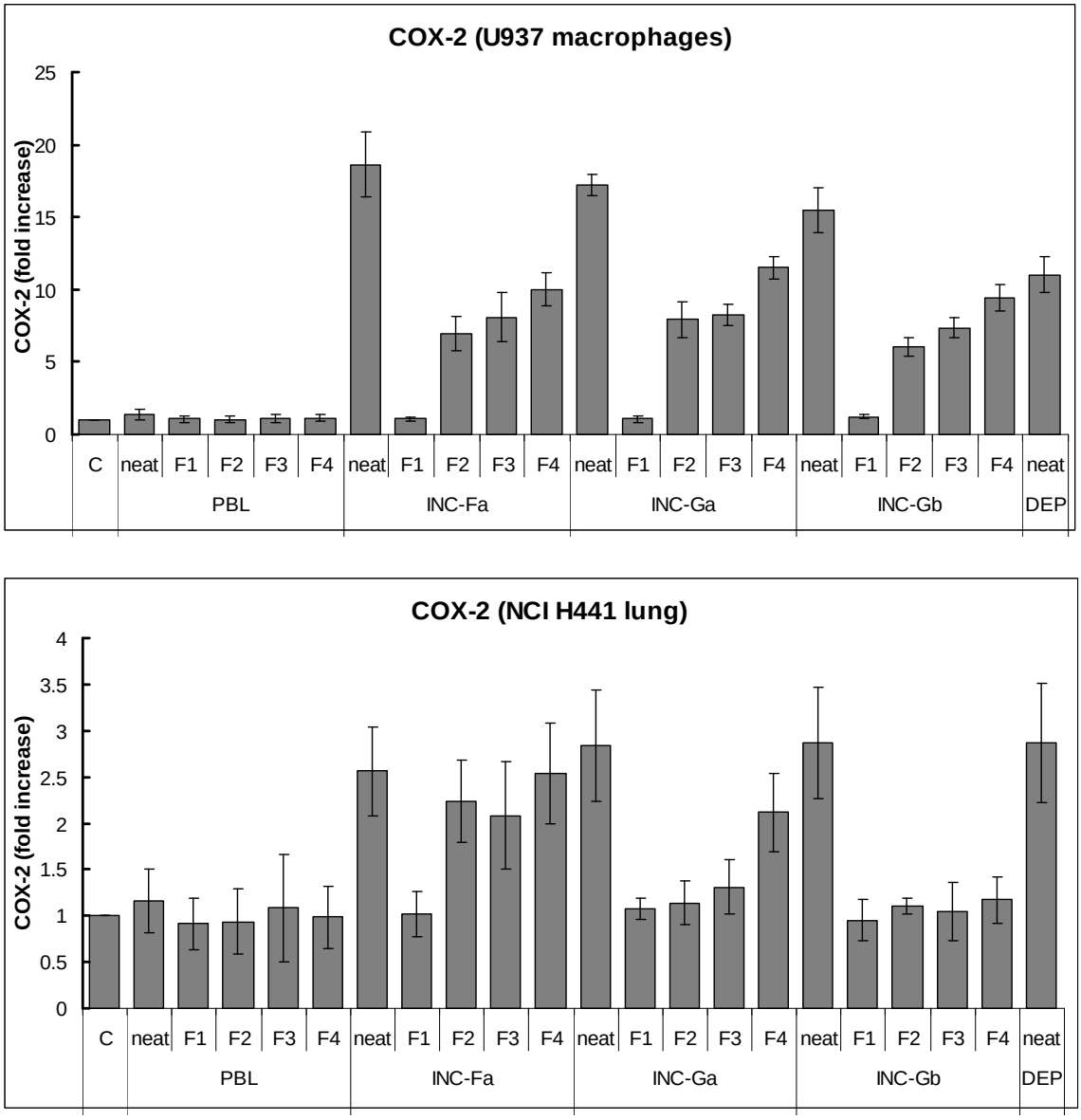


Figure 4-3. Effect of extracts from three indoor source incense PM samples on cyclooxygenase 2 (COX-2) mRNA expression in U937 macrophages and NCI H441 Clara lung cells.
Error bars represent mean ± SD of triplicate determinations.
Sample ID and information see Figure 4-2.

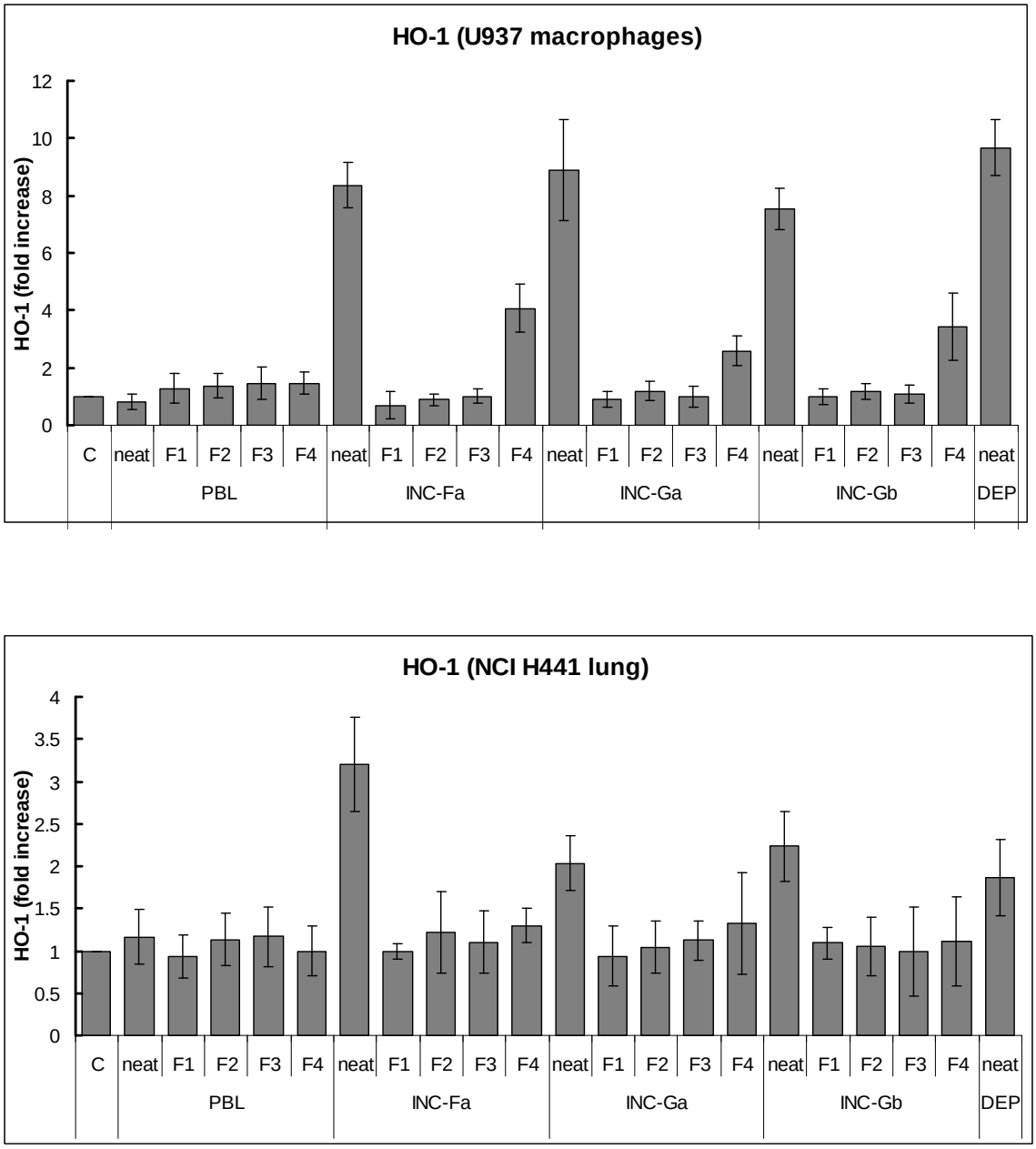


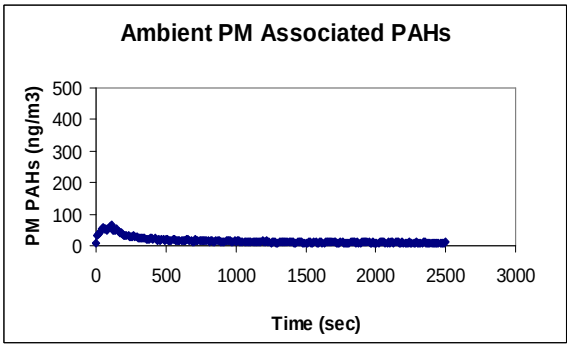
Figure 4-4. Effect of extracts from three indoor-source incense PM samples on heme oxygenase-1 (HO-1) mRNA expression in U937 macrophages and NCI H441 Clara lung cells.
Error bars represent mean ± SD of triplicate determinations. Sample ID and information see Figure 4-2.

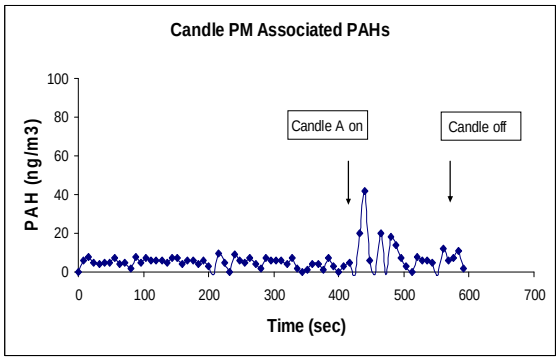
4.3 PAHs in Indoor PM Source Samples

4.3.1 Initial Screening: Real-time monitoring of PAHs

An initial chemical survey was conducted using the EcoChem PAS PAH sampler (Ecochem Analytics, League City, TX) to see if particle-associated PAHs could be detected in selected samples. The PAS instrument detects the PAHs associated with the particles and reports the concentrations in nanograms (ng)/m^3. Candles, woodsmoke and incense were measured as indoor source PM using the instrument. The results are summarized in Figure 4-5 for candles.

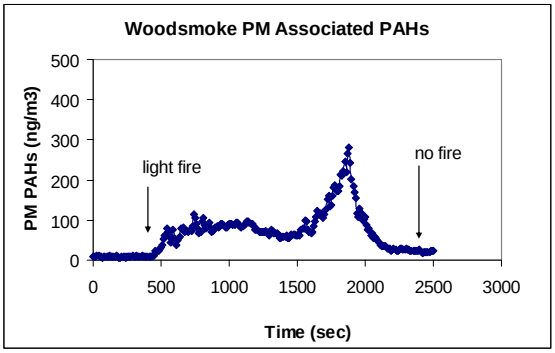
The peak levels of PAHs were around 20 to 40 ng/m^3. For woodsmoke, the values were considerably higher as seen in Figure 4-5. The first burn event is illustrated in the left graph and the second burn event illustrated in the right graph. The different burns had maximum peak levels of 300 to 700 ng/m^3. The indoor air PAH sample without wood being burned is presented in Figure 4-5. The levels are low. The PAH concentrations measured for incense are presented in Figure 4-5. Here we see that for incense samples tested, there were considerable levels of PAHs. The PAH concentrations peaked at around 2,000 ng/m^3. The PAS sampler provided a chemical screening and further quantitative chemical analyses of the PAHs in the indoor PM sample extracts.

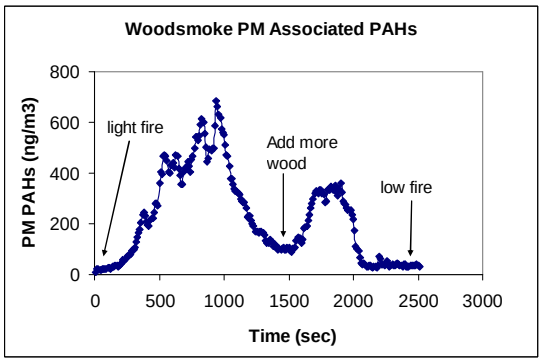




. First Burn

Second Burn





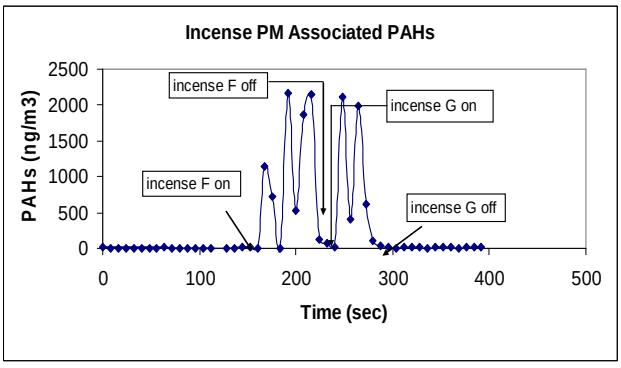


Figure 4-5. Initial readings from ambient air, candles, woodsmoke, and incense samples using a Ecochem PAH monitoring instrument (PAS) Instrument. The woodsmoke diagrams represent two separate burns. Note the Y axis scale for the incense sample.

4.3.2 Quantitative Chemical Analyses of PAHs

Quantitative PAH analyses were conducted for the extracts that were used for the biological assays. Gas chromatography/mass spectral (GC/MS) methods previously published for diesel and heavy-duty engine emission exhaust PM (Okamoto et al., 2006; Kado et al., 2005) were employed. Briefly, a Hewlett-Packard (HP) 5890 Series II gas chromatograph interfaced to a HP5972 mass selective detector run in selective ion monitoring mode (SIM) was used throughout. The injector was operated in splitless mode. The GC was equipped with a DB-5ms fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with electronic pressure control. PAH standard reference material SRM 2260 (NIST, Gaithersburg, MD) was used to prepare calibration solutions. Additionally, a limited number of XAD samples were also analyzed for PAHs. Briefly, XAD samples were extracted in DCM four times by shaking in separatory funnels. To remove interfering compounds, extracts were subjected to silica fractionation to isolate the PAH fraction. The silica fractionation was conducted in the same manner as for the incense fractionation experiment described above. The F2 fractions (PAH fraction) were analyzed for PAHs.

The PAH levels are summarized in Table 4-1 for cooking samples and Table 4-2 for the incense sample. Due to the limited amounts of PM collected, PAHs were detected only in trace levels and many were not quantifiable in candle, cooking, and woodsmoke samples. The results for the candle and woodsmoke samples are presented in Table A-1 and A-2 in the appendices. In one of the woodsmoke samples, benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene were detected at the levels slightly above the detection limit. In a XAD sample for the woodsmoke, a number of PAHs were found at levels above the corresponding background air sample, including naphthalene, methyl-naphthalenes, acenaphthylene, fluorene, and phenanthrene. This is consistent with the study by McDonald et al. (2000), who reported on the chemical characterization of woodsmoke from a fireplace using a dilution stack sampler. The authors also reported levels of vapor-phase PAHs including naphthalene and methyl-naphthalenes,

In spite of the small amount of PM collected, a number of PAHs were quantified in the incense sample. Fluoranthene, pyrene, benz(a)anthracene, chrysene+tripheylene, benzo(b) and benzo(k)fluoranthenes, for example, were quantified. Benzo(a)pyrene and benzo(e)pyrene had interfering compounds that co-eluted with these so quantitation was not possible. Heavier PAHs such as indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene were also detected. The PAH concentrations in ng/m^3 determined in the incense PM extract were comparable to the PAS data measured for the incense sampling. The levels of PAHs in ng/mg PM quantified in the incense sample were some of the highest that we have ever seen, and were comparable or possibly even higher than PAH levels in diesel PMs that have been measured in our laboratory. The high PAH concentrations found in the incense PM is consistent with our

finding of molecular markers such as CYP1A1 which was present at higher levels than diesel engine particle standard (SRM 2975) that was used as a positive control in the biological testing. PAHs emitted from incense burning have been studied as a significant human health risk in Asia (Chiang et al., 2009). As the use of incense is getting more popular in California, more research would be needed in the area of exposure and risk assessment associated with incense burning.

Table 4- 1. PAHs identified in the cooking samples.

PAH	PM10 Filter samples (ng/mg PM)		XAD samples (ng/m^3 air)		
	CK-1 Stir-fry	CK-2 Oven	Stir-fry	Oven	Background air
Naphthalene	<0.3	<30	**11**	**23**	**22**
2-Methylnaphthalene	C.E.	<30	**23**	**30**	**20**
1-Methylnaphthalene	<0.3	<30	**12**	**14**	**9.2**
Biphenyl	<0.3	<30	<3	**4.6**	<3
2,6-Dimethylnaphthalene (coelute)	<0.3	<30	<3	**5.3**	**3.7**
Acenaphthylene	<0.3	<30	<3	<3	<3
Acenaphthene	<0.3	<30	C.E.	C.E.	C.E.
2,3,5-Trimethylnaphthalene (coelute)	<0.3	<30	<3	<3	**4.0**
Fluorene	<0.3	<30	C.E.	C.E.	C.E.
Phenanthrene	<0.3	<30	<3	<3	<3
Anthracene	<0.3	<30	C.E.	C.E.	<3
1-Methylphenanthrene	<0.3	<30	<3	<3	<3
Fluoranthene	<0.3	<30	<3	<3	<3
Pyrene	<0.3	<30	<3	<3	<3
Benz(a)anthracene	<0.3	<30	<3	<3	<3
Chrysene+triphenylene	<0.3	<30	<3	<3	<3
Benzo(b)fluoranthene	<0.3	<30	<3	<3	<3
Benzo(k)fluoranthene	<0.3	<30	<3	<3	<3
Benzo(e)pyrene	<0.3	<30	<3	<3	<3
Benzo(a)pyrene	<0.3	<30	<3	<3	<3
Perylene	<0.3	<30	<3	<3	<3
Indeno(1,2,3-cd)pyrene	<0.3	<30	<3	<3	<3
Dibenz(a,h)anthracene	<0.3	<30	<3	<3	<3
Benzo(g,h,i)perylene	<0.3	<30	<3	<3	<3

C.E. = co-elution of interfering compounds in the complex mixture.
No PAHs were detected in the matched background air filter sample.

Table 4- 2. PAHs identified in the PM incense sample

PAH	Incense Sample ID: INC-G (ng/mg PM)
Naphthalene	<0.2
2-Methylnaphthalene	**1.6**
1-Methylnaphthalene	**1.3**
Biphenyl	**10**
2,6-Dimethylnaphthalene (coelute)	<0.2
Acenaphthylene	**17**
Acenaphthene	<0.2
2,3,5-Trimethylnaphthalene (coelute)	<0.2
Fluorene	C.E.
Phenanthrene	C.E.
Anthracene	C.E.
1-Methylphenanthrene	C.E.
Fluoranthene	**35**
Pyrene	**31**
Benz(a)anthracene	**12**
Chrysene+triphenylene	**24**
Benzo(b)fluoranthene	**13**
Benzo(k)fluoranthene	**2.7**
Benzo(e)pyrene	C.E.
Benzo(a)pyrene	C.E.
Perylene	C.E.
Indeno(1,2,3-cd)pyrene	**3.4**
Dibenz(a,h)anthracene	**1.6**
Benzo(g,h,i)perylene	**2.9**

Incense = PM2.5, Sample ID INC-G (Floral Variety)
C.E. = co-elution of interfering compounds in the complex mixture

4.3.3 Qualitative Chemical Characterization

Cooking Samples

The total ion current chromatogram (TIC) by GC/MS electron impact mode was obtained for cooking sample extracts and the mass spectrum for major peaks were compared with the NIST library to identify the compounds. The GC column and the GC conditions such as injection and oven temperatures were the same as for the GC/MS operation under SIM as described above. The extracts investigated were the ones that were tested by the biological tests. The PM equivalents used for the TIC analyses were dependent on the PM collected and are indicated in the TIC figures illustrated in the appendices.

Approximately 14 µg of PM for the stir-fry (CK10-1) and 0.1 µg for the oven cooking (CK10-2) were analyzed. The quantity used for the TIC analysis for the oven cooking sample was small due to low amounts of particles collected. TICs from the GC/MS analyses of the extracts of stir-fry and oven cooking filter samples were obtained and presented in Figure B1 and B2 in the Appendix. Major peak identifications are also listed in the appendices under each figure.

The major peaks identified in the stir-fry sample was unsaturated hydrocarbons such as heptadecene and squalene, carbonyls including methacrolein, pentadecanal, hexadecenal, octadecenal, hexadecanoic acid, octadecenoic acid methyl ester, oleic acid and octadecanoic acid, saturated hydrocarbons such as pentacosane, and phenolics such as γ-tocopherol. Many of these compounds are thought to be in the cooking ingredients, especially in peanut oil, or the oxides of the ingredients. The highest peak in the chromatogram was oleic acid (unsaturated fatty acid, major component of peanut oil), followed by squalene (unsaturated hydrocarbon, also contained in peanut oil), and campesterol and stigmasterol (plant sterols, contained in peanut oil). Compared to the stir-fry cooking sample, the abundance of the chemicals found in the oven cooking was very low (much less than 10%) partly because of the low amount of PM sample available and the low amount of PM equivalent injected to the GC. In the oven sample, very few peaks were present except for the ones that we observed in other samples including background air samples and process blank. The compounds for these peaks most likely are from the process of sampling, extraction or analysis and may be siloxanes.

Candle Samples

The TIC was obtained for the extract from a candle (CN-B) by extracting it in DCM by shaking and sonication for the biological analyses as described earlier. The PM equivalent injected to the GC was approximately 2.4 µg. TIC of the candle DCM extract is also illustrated in Figure B3. Sharp peaks observed in the

chromatogram were identified as long-chain alkanes and alkenes such as decyl-tetracosane (C34) and nonadecene (C19).

Fine et al. (1999) chemically characterized fine PM emissions from burning church candles in a chamber study. They reported that the majority of emissions were organic compounds that included alkanes, alkenes, alkanoic acids, wax esters, and cyclohexylalkanes. The PM size ranged from less than 0.1 µm during "normal burning" to less than 1 µm during "smoking" and "smoldering" phases.

Woodsmoke

The TIC for woodsmoke PM 10 sample is illustrated Figure B-4. The major peaks observed in the TICs for the woodsmoke samples are all thought to be siloxanes, compounds that are composed of unit of the form R_2SiO where R is a hydrogen atom or a hydrocarbon group. The origin of these compounds is not clear but they may have been added. For example, siloxanes can be used to water-proof wood. The firewood was seasoned almond variety, reported by the dealer to have been aged for at least 1 year. These were not manufactured fire logs that can be coated with waxes. Any other classes of compounds were not positively identified in this sample.

McDonald et al., (2000) reported a number of hydrocarbons for the burning of hardwood where emissions were collected using a dilution stack sampler. Of the PM-associated hydrocarbons they reported compounds such as PAHs, guaiacols (aromatic oil in wood), and sesquiterpenes (class of terpenes), for example.

Incense

Variety Pak (ID INC-G)

For the incense samples, chemical composition of the four fractions that were examined for the biological potency were individually investigated. TICs of the four fractions from incense (INC-Ga) "floral India" are presented in Figures B-5 through B-8. The PM equivalent injected was approximately 40 to 50 µg PM for these fractions. Note the scales for the Y-axis are very different for different fractions where in F4 it is more than an order of magnitude higher than in F3 and almost 3 orders of magnitude higher than in F2 and F1.

In F1, again the same peaks were observed as in the oven cooking sample, the second fraction of candle and background air, and the oven cooking sample, which are most likely siloxanes. Some of the other compounds present in very small quantities may be alkanes. In F2, some PAHs such as phenanthrene, anthracene, and fluoranthene were identified in this fraction. Also, dibenzofuran

appeared to be present in this fraction. In F3, compounds found in high concentrations included a number of carbonyl compounds such as 2-phenylmethylene-octanal (alpha-hexylcinnamaldehyde), 2-phenylmethyleneheptanal (amylcinnamaldehyde), Lilial (p-*tert*-butyl-*alpha*-methyl-hydrocinnamaldehyde), benzyl benzoate, and 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin. These compounds are generally used as flavoring agents for perfume and cosmetics. In F4, the sharp peak observed at R.T. 17.7 min with very high response was identified as ethyl vanillin, which is a synthetic compound commonly used as a flavorant in food and perfumes. There was a large hump after R.T. 35 min in F4 that was not chromatography separated. Some of the possible compounds for this hump are substituted nitrophenols and substituted bromobenzenes. Other compounds identified between R.T. 17.7 to 35 min were some fatty acids and their esters.

It has been reported that ethyl vanillin, a compound found in large quantity in F4, and cinnamic aldehydes found in F3 may be skin irritants (Basketter et. al., 2001; Arts et. al., 2006). It is possible that these compounds may be contributing to the high potency of F3 and F4 of the incense sample toward COX-2. We provide an example of the TIC for fraction 4 in Figure 4-6 with a partial list of compounds characterized. The full list along with the TICs for all fractions are presented in the appendices.

Navasurmrit et al. (2008) reported high exposure of temple workers to benzene,1,3-butadiene and PAHs relative to control workers. Biomarkers of exposure to these compounds were significantly higher in temple workers than in control workers and temple workers also had a 2-fold increase in DNA damage as DNA strand breaks in leukocytes.

Incense (INC-F Joss Stick)

Japanese Buddhist incense sample INC-F (manufactured by Mainichi-koh) was extracted by pressurized solvent extractor (ASE200, Dionex) in DCM. The DCM extract was solvent exchanged to hexane and fractionated in the same manner as for Incense Floral as described above. TICs of these fractions are presented in Figures B-9 through B-13. The PM equivalent mass analyzed in the GC/MS was 2 to 2.3 µg PM for each fraction. Note the scales for Y-axis are again very different for different fractions where Y-axis scale in F3 and F4 was an order of magnitude higher than the one in F1 and F2. The chromatographic response observed in F1 and F2, in general was approximately 10% of F3 and F4.

In F1 some of the major peaks were characterized as siloxanes, which may be ingredients of the incense, the GC column bleed or something from the sampling and extraction processes. There were a number of additional peaks, but identification could not be made for these peaks. F2 TIC looks very similar to

the second extracts of a candle and the background air, the oven cooking sample, and the F1 from the incense (INC-G), where major peaks were siloxanes. In addition, 2-methoxy naphthalene was identified in this fraction, which may be an ingredient of manufacturing the incense. F3 contained compounds that were higher amounts than F1 or F2. The largest peak was identified as benzyl benzoate, which may be an ingredient of manufacturing the incense or a component of a natural products (for example, plants) used in incense. The other compounds observed were carbonyls and again some siloxanes. In F4 the largest peak was identified as vanillin (4-hydroxy-3-methoxybenzaldehyde), which may be an ingredient of the incense and can be natural. Other compounds in this fraction include some fatty acids (such as octadecanoic acid), esters (such as hexadecanoic methyl ester), ketones, phenolic compounds, and again siloxanes.

Although the biological profile among these fractions were similar between the Buddhist incense (INC-F) and the Floral incense (INC-G) and where the polar fraction was more potent than semi-polar or non-polar, the TICs were markedly different in these two incense samples. Further research in characterizing the incense PM in larger quantities will help to better understand the mechanism of high toxicity of incense PM.

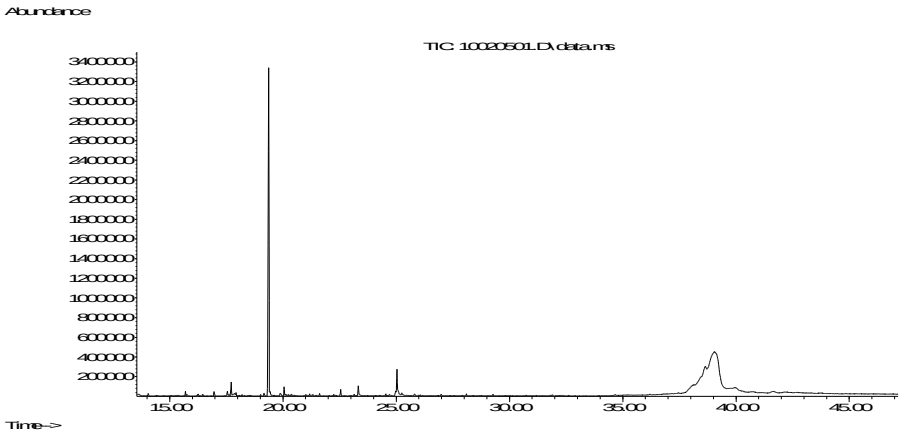


Figure 4-6. Total Ion Chromatogram of Fraction 4 from Incense sample. Provided as an example of TICs. Complete sets of TICs are in the Appendix. Partial list of compounds tentatively identified in the sample are shown below with a more complete list presented in the appendices.

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	14.05	0.17	C:\Database\NIST05.L			
			Benzoic acid, 2-hydroxy-, methyl ester	24827	000119-36-8	95
			Benzoic acid, 2-hydroxy-, methyl ester	24832	000119-36-8	93
			Benzoic acid, 2-hydroxy-, methyl ester	24831	000119-36-8	93
2	15.696	0.33	C:\Database\NIST05.L			
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	91
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	87
			Cyclohexasiloxane, dodecamethyl-	179151	000540-97-6	47
3	15.757	0.23	C:\Database\NIST05.L			
			2-Propen-1-ol, 3-phenyl-	14809	000104-54-1	91
			2-Propen-1-ol, 3-phenyl-	14811	000104-54-1	70
			2-Propen-1-ol, 3-phenyl-	14804	000104-54-1	64
4	16.265	0.25	C:\Database\NIST05.L			
			Phenol, 2,6-dimethoxy-	26272	000091-10-1	94
			Phenol, 2,6-dimethoxy-	26275	000091-10-1	93
			Phenol, 3,4-dimethoxy-	26273	002033-89-8	74
5	16.465	0.15	C:\Database\NIST05.L			
			2(3H)-Furanone, dihydro-5-pentyl-	27819	000104-61-0	72
			2(3H)-Furanone, dihydro-5-pentyl-	27812	000104-61-0	72
			2(3H)-Furanone, dihydro-5-pentyl-	27818	000104-61-0	64
6	16.957	0.34	C:\Database\NIST05.L			
			Vanillin	24743	000121-33-5	98
			Vanillin	24745	000121-33-5	97
			Vanillin	24742	000121-33-5	96
7	17.542	0.48	C:\Database\NIST05.L			
			2H-1-Benzopyran-2-one	21396	000091-64-5	93
			2H-1-Benzopyran-2-one	21395	000091-64-5	89
			2H-1-Benzopyran-2-one	21397	000091-64-5	76
8	17.634	0.16	C:\Database\NIST05.L			
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl -3,5,5-tris(trimethylsiloxy) tetra siloxane	187800	071579-69-6	32
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	27
			Pentasiloxane, dodecamethyl-	166196	000141-63-9	27
9	17.711	1	C:\Database\NIST05.L			
			Ethyl Vanillin	33932	000121-32-4	97
			Ethyl Vanillin	33930	000121-32-4	97
			Ethyl Vanillin	33933	000121-32-4	96

5. DISCUSSION AND CONCLUSIONS

5.1 Introduction

Exposure to airborne particulate matter (PM) is a health concern for the people of California, since much of the health effects from exposure to air pollution are hypothesized to be related to PM exposure. Indoor air pollution is of concern since people are reported to spend approximately 80 to 90 percent of their time indoors and there are potentially serious indoor sources of air pollutants including PM. Exposure from PM sources indoors can occur near the source. A report to the California Legislature prepared by the Air Resources Board recommends that indoor pollutants and their sources should be ranked in the high-risk category (CARB 2005).

In the current study, methods were developed to integrate a number of indoor source particulate matter with human cell systems. A number of indoor source PM were evaluated for their potential to induce inflammatory cellular response including oxidative reactions in cells derived from the respiratory system. To our knowledge, the current study is one of the few that have integrated a number of indoor source PM samples with markers of inflammation in human cells. Some of the highest indoor PM levels are derived from combustion processes such as smoking, cooking, and burning of wood and candles, and also from cleaning activities (Wallace *et al.*, 2003; Ozkaynak *et al.*, 1996a,b; Brauer *et al.*, 2000; Abt *et al.*, 2000, 2001; Fortmann *et al.*, 2001; Long *et al.*, 2000). The activity of cooking and the burning of candles, for example, also produce ultrafine PM (Buonanno et al., 2009; Wright et al., 2007). Since exposure is potentially elevated due to proximity to the source generating PM, we evaluated procedures to sample and then test the PM in human cell bioassays.

5.2 Evaluation of bioassay cell systems

Our initial study found that the human cell line A549 had limited sensitivity to detect the toxicity and inflammatory potential of indoor PM from various sources except for incense. On the other hand, the U937 derived human macrophages appear sensitive, resulting in elevated levels of COX-2 as well as CYP1A1, towards the exposure of various indoor PM sources. The results confirm our earlier findings (Vogel et al. 2005) with standard reference PM and positive control compounds, and show that the U937 macrophage cell line is a suitable *in vitro* model for indoor PM testing. Two additional human cell systems were evaluated in initial studies and it was found that epithelial cell line HPL-1 was less sensitive than Clara cell line H441 for the indoor PM. H441 cell line is derived from a human bronchiolar Clara cell which is a non-ciliated epithelial cell line

present as a major cell type on the surface of small (< 2 mm) airways. In conclusion, a human macrophage cell line (U937) and the Clara cell line were thought to have adequate sensitivity to be used throughout the study.

5.3 PM Source Samples

A number of indoor PM source samples of cooking, candle burning, wood burning, and incense burning, were collected and tested in the cell system. Each source is discussed.

Cooking - Cooking samples from stir-fry increased CYP1A1 expression in macrophages. The oven cooking PM had no significant effect on CYP1A1 expression in macrophages. However, both cooking samples from stir-fry and oven cooking increased the expression of inflammatory marker COX-2 as well as IL-8 in macrophages. For COX-2, stir-fry was about 4-fold over control, while oven cooking resulted in about 5-6 fold increase. For IL-8, stir-fry was about 10-fold, while oven was about 9-fold over control levels. The only significant effect in NCI H441 cells was found on MUC5AC expression by treatment with extract from stir-fry cooking sample. Higher concentrations of PM and a variety of cooking conditions would probably evoke greater responses. While the oven cooking sample has effects on COX-2 and IL-8 in U937 macrophages only, the extract of the stir-fry cooking PM generated significant effects on CYP1A1, COX-2, IL-8 in macrophages and MUC5AC in NCI H441 cells. These results for stir-frying are consistent with the PM2.5 levels and compounds measured reported in the kitchen after stir-frying by Fortmann et al., 2001 and See et al., 2008. In conclusion, stir-fry cooking and oven baking resulted in detectable levels of the expression of inflammatory markers and oxidative reactions.

Candles - In a series of candle samples, there was initially low potential for inducing CYP1A1 and the inflammatory markers in the cells tested. Further, no significant change was observed on the expression of HO-1 in both cell lines. However, subsequent testing found that test candle CN-C had a very high level of CYP1A1 expression – over 16-fold over background. This candle also had increased COX-2 and IL-8 expression. The PM sample from CN-B had a slight effect (3-fold) on the expression of CYP1A1. In conclusion, the results show that PM from some candles like CN-C may contain a sufficient amount of PAHs or other compounds which may lead to the induction of the AhR-regulated gene CYP1A1 and inflammatory marker genes COX-2 and IL-8. Regarding the emission of ultrafine particles, Afshari et al., (2005) reported that the highest observed concentration of ultrafine particles was from pure wax candles (2.4×10^5 particles/cm^3).

Woodsmoke – Woodsmoke samples increased CYP1A1 and COX-2 expression in macrophages. Some of the woodsmoke samples tested had significant effect on CYP1A1 and COX-2 expression both in macrophages and in

the human NCI H441 cells. PM2.5 and PM10 samples were taken in parallel for two wood burning events. Both PM10 and PM2.5 from burn #2 induced CYP1A1 both in macrophages and in NCI H441 lung cells. The induction levels in the lung cells were higher than DEP used as a positive control and tested in parallel to the woodsmoke samples. For burn #2, induction of CYP1A1 was observed only in macrophages for PM2.5. PM10 from burn #2 did not show any elevated level of CYP1A1 above the background air in either macrophages or the lung cells. Similar results were obtained for the inflammatory marker gene COX-2 in macrophages where both PM10 and PM2.5 from burn #2 had effects but for burn #1 only PM2.5 had an effect. These samples also showed induction of COX-2 in macrophages. In the lung cells the induction of COX-2 was weak for both PM10 and PM2.5. None of the woodsmoke samples tested had any effects on MUC5A in the lung cells. In conclusion, the woodsmoke PM sample acquired appears to have significant effects on toxicity and the inflammatory marker genes like CYP1A1, COX-2 and IL-8, but not MUC5AC. These findings are consistent with the literature from both in vitro and in vivo toxicologic studies (Naeher et al, 2007) where inflammation and oxidative stress may be one of the modes of toxicity.

Incense - All incenses tested were very active in the induction of CYP1A1, IL-8, and COX-2. The expression of HO-1 used as an indicator for oxidative stress and MMP-12 (atherogenic marker for cholesterol-accumulating macrophages) was significantly increased also but not as strongly as CYP1A1, IL-8, or COX-2. The expression levels of CYP1A1, COX-2, and IL-8 in macrophages were increased in a dose-dependent manner. Markers for CYP1A1, COX-2, and MUC5AC also were increased in a dose-dependent manner in NCI H441 cells. A detectable increase of all three marker genes was found at the lowest concentration (1 µg/ml) tested. Although the incense samples overall were strong inducers for inflammatory markers there were differences in the levels of induction among different incense samples.

In conclusion, dose-response relationships were developed for incense samples for a number of the markers. In view of the exceptionally high increase of inflammatory markers elicited by relatively low concentrations of incense PM (1 µg/ml) and high level of AhR potency and the presence of polycyclic aromatic hydrocarbons (PAHs) (see chemical analysis), combined with a high radical-generating capacity indicated by significant increase of HO-1 expression, it cannot be excluded that regular exposure to incense-derived PM results in increased risk of inflammatory effects, which in turn could result in pulmonary or cardiovascular effects.

5.4 Biological and Chemical Characterization of PM

Bioassay-Directed PM Fractionation - The PM samples with the highest responses were further investigated to provide insight into mechanisms of toxicity. The incense samples were chosen for this study due to their much

stronger activities in inducing toxicity and inflammatory or oxidative stress markers compared to the PM samples of other sources. The incense samples were chemically fractionated by eluting through silica using solvents with different polarities. Fraction 1 (F1) was most non-polar (eluting in hexane), followed by fraction 2 (F2) and then fraction 3 (F3), with slightly more polar mixtures of hexane and dichloromethane, and the last fraction (F4) was most polar, eluting in methanol. Preliminary experiments with PAH and nitro-PAH standard chemicals found that PAHs elute in F2 and nitro-PAHs are in F2 and F3. These fractions were tested in the two human cell systems for CYP1A1, COX-2 and HO-1 expression. Cells were treated with10 µg/ml PM equivalent extract from 3 different incense samples for 24 hr. We tested the total extract or raw/neat extract of each incense sample and their corresponding fractions from non-polar to polar compounds. In both cell lines, U937 macrophages and NCI H441 cells, we found the highest increase of CYP1A1 after treatment with the raw or neat (un-fractionated) material, followed by the most polar fraction 4 (F4) of the three incense extracts tested. The effect of F3 and F2 was also significant but not as strong as the effect of F4 at the same concentration. Treatment with the most non-polar fraction F1 had no significant effect on the expression of CYP1A1 in both cell lines tested. In conclusion, in all cases, the most active fraction was in the most polar fraction.

Chemical Investigation of PAHs – The initial investigation of real-time monitoring of PAHs was conducted for candle, woodsmoke, and incense sampling. Based on particle associated PAHs detected by the real-time monitor, the peak levels of PAHs for a candle sample was around 20 to 40 ng/m^3. For woodsmoke, the values were considerably higher for different burns, with maximum peak levels of 300 to 700 ng/m^3. The indoor air PAH levels prior to wood burning were low. The PAH concentrations measured for incense peaked at around 2,000 ng/m^3. The real-time monitor provided a chemical screening, and further quantitative chemical analyses of the PAHs were conducted using GC/MS for representative samples from all sources investigated. Based on the PM collected using low volume air sampling, many of the PAHs were detected only in trace levels and most of them were not quantifiable for all the samples except for one woodsmoke sample and one incense sample. PAHs were quantitatively measured at levels slightly above the detection limit for the woodsmoke sample. For example, fluoranthene, pyrene, benz(a)anthracene, chrysene+triphenylene, benzo(b) and benzo(k)fluoranthene, were quantified in the incense sample. Heavier PAHs such as indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene were also detected. Concentrations of these compounds were comparable or possibly higher than diesel PM samples tested in our laboratory. The quantitative chemical analysis of PAHs result supported our biological finding where the incense samples induced CYP1A1 at even higher levels than the diesel engine particles.

Chemical Investigation of Additional Compounds – Total ion chromatography (TIC) for the representative samples from all sources were obtained to investigate the presence of compounds other than PAHs. TIC and major compounds present in the samples were different in samples from the different sources. In cooking samples, a number of compounds such as unsaturated hydrocarbons heptadecene and squalene, carbonyls including methacrolein, were present in high levels and were thought to be from peanut oil that was used for the stir-fry cooking. The TIC of the oven sample looked similar to an ambient air sample and is probably due to the low sample amount that was collected. Major compounds found in a candle samples were saturated and unsaturated long-chain hydrocarbons. The major compounds found in a woodsmoke sample were a number of siloxanes, which possible was contained in the wood. TIC of the incense sample was obtained from the fractionation experiment. Fraction 4 (F4) of the incense samples (INC-F and INC-G), was the most polar fraction and most active in the biological tests had compounds in much higher quantity than the non-polar fraction. In this fraction, a flavoring agent, vanillin, was found in the largest quantity, which may be contributing to some of inflammatory response due to the evidence reported in literature as a skin irritant. The TIC profiles of these fractions were different in two different incenses in terms of major chromatographic peaks present in each fraction. Although these incense samples had similar trends in human cell assays (F1 through F4), and the most active fraction in the bioassays was the polar fraction, the contributing components may differ in these two incenses. Both incense samples had a number of carbonyl compounds present in lesser quantities in F3 and F4. It also is possible that the activities collectively come from these compounds.

In summary, most of the indoor source particulate matter tested had responses in at least one of the human cell lines and marked differences were found in the activities from the indoor source PM collected. For example, the incense PM sample induced higher responses than did the other indoor source PM samples. Further, the bioassay-directed chemical analysis approach with the incense samples showed that the polar fractions were the most active. The objectives of the integrated study were met with additional information generated from the diverse group of samples acquired from cooking, candle, woodsmoke, and incense samples. The study consistently found that incense burning generates PM with high inflammatory or oxidative activities and high levels of PAHs. As incense use is becoming more common in California, further research toward exposure and risk analysis for incense burning would be important. However, although their activity levels may appear lower, from a public health perspective, attention should also be paid to candles and cooking since the PM generated from these indoor sources induced inflammation and oxidative stress markers and these indoor PM sources are probably more ubiquitous.

6 RECOMMENDATIONS

Four recommendations are offered as a result of this study.

First, although the incense was the most potent PM of the source PM studied, all of the PM sources induced the inflammatory markers. The different PM source samples also had different levels of activity. It is recommended that a larger survey of these samples, especially for cooking and candles, using the bioassay approach, would provide a robust database for these indoor PM sources.

Second, further development of bioassay-directed chemical analysis for the cooking and candle PM would provide directions in what components are responsible for these activities. Once components are isolated, strategies to mitigate exposure could be developed.

Third, limited parallel *in vivo* studies can be developed for selected indoor PM in parallel to the bioassays presented in the current report to bridge toxicity information.

Fourth, chemical characterization approaches of the PM would provide information to the ARB and manufacturers for use, handling, and exposure to potentially toxic compounds. Some of the indoor source PM samples have high levels of PAHs, a group of toxic air contaminants; and there are other components in the PM that are very active in inducing inflammatory and oxidative responses in the human cell systems tested.

6.1 Benefits to California

The current study provided methods that integrate indoor source PM samples and bioassay analyses using inflammatory marker and oxidative stress responses in human cells. This approach will help the ARB in the assessment of exposure and health effects evaluation of indoor PM sources.

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8. GLOSSARY, ABBREVIATIONS

AhR: Aryl hydrocarbon receptor

BaP: Benzo(a)pyrene

CB: Carbon black

cDNA: Complementary DNA

COX-2: Cyclooxygenase 2

CRP: C-reactive protein

CSE: Cigarette smoke extract

CVD: Cardiovascular disease

CYP1A1: Cytochrome P4501A1

DCM: Dichloromethane

DEP: Diesel exhaust particulate (NIST reference diesel particulate matter, SRM 2975 or SRM 1650)

D-MEM:Dulbecco's Modified Eagle Medium for cell culture

DMSO: Dimethyl sulfoxide

DNase: Deoxyribonuclease

FBS: Fetal Bovine Serum

FcγR: Fcγ receptor

GADPH: Glyceraldehyde-3-phosphate dehydrogenase

GC/MS: Gas chromatography/mass spectrometry

HEPA: High efficiency particulate adsorbing (filter material)

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer

HO-1: Heme oxygenase-1

IgG: Immunoglobulin G

IL: Interleukin (IL)

LDL: low density lipoprotein

MCP-1: Monocyte chemoattracting protein-1

MMP: Matrix metalloproteinase

MNF: Antagonist of the AhR

mRNA: Messenger RNA

MUC5AC: Mucin 5AC

Nitro-PAHs: Nitrated polycyclic aromatic hydrocarbons

NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells – controls transcription of DNA

NIST: National Institute of Standards & Technology

OE-DEP: Organic extracts of diesel particles

OE-UDP: Organic extracts of urban dust

PAHs: Polycyclic aromatic hydrocarbons

PAS: Photoelectric aerosol sensor (EcoChem Analytics)

PBS: Phosphate buffered saline

PCR: Polymerase chain reaction

PM: Particulate matter air pollution

PM2.5: Particles with aerodynamic diameter less than or equal to a nominal 2.5 microns

PM10: Particles with aerodynamic diameter less than or equal to a nominal 10 microns

RT-PCR: reverse-transcriptase-polymerase-chain-reaction

PUF: Polyurethane foam – used as an adsorbent for airborne vapor-phase compounds

RIPA: Radioimmunoprecipitation assay – buffer for cell lysis

RNase: Ribonuclease

RPMI: Roswell Park Memorial Institute - solution used for cell culture

sDEP: Stripped particles of diesel exhaust particles

SP-A: Pulmonary surfactant protein-A

SRM: Standard Reference Material - NIST

sUDP: Stripped particles of urban dust

TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

TNFα: Tumor necrosis factor α

TPA: 12-O-tetradecanoylphorbol-13-acetate

UDP: Urban dust particles (NIST Reference material 1649a urban particulate matter)

UV: Ultraviolet light (wavelength shorter than that of visible light)

VEGF: Vascular endothelial growth factor

XAD: Polystyrene-divinylbenzene resin used as adsorbent for airborne vapor phase

Appendix A. PAH concentrations for candle and woodsmoke samples

Table A- 1 PAHs identified in the candle samples

PAH	Candle CN-A (ng/mg PM)
Naphthalene	<1.4
2-Methylnaphthalene	<1.4
1-Methylnaphthalene	<1.4
Biphenyl	<1.4
2,6-Dimethylnaphthalene (coelute)	<1.4
Acenaphthylene	<1.4
Acenaphthene	<1.4
2,3,5-Trimethylnaphthalene (coelute)	<1.4
Fluorene	<1.4
Phenanthrene	<1.4
Anthracene	<1.4
1-Methylphenanthrene	<1.4
Fluoranthene	**1.9**
Pyrene	**1.9**
Benz(a)anthracene	<1.4
Chrysene+triphenylene	<1.4
Benzo(b)fluoranthene	<1.4
Benzo(k)fluoranthene	<1.4
Benzo(e)pyrene	<1.4
Benzo(a)pyrene	<1.4
Perylene	<1.4
Indeno(1,2,3-cd)pyrene	<1.4
Dibenz(a,h)anthracene	<1.4
Benzo(g,h,i)perylene	<1.4

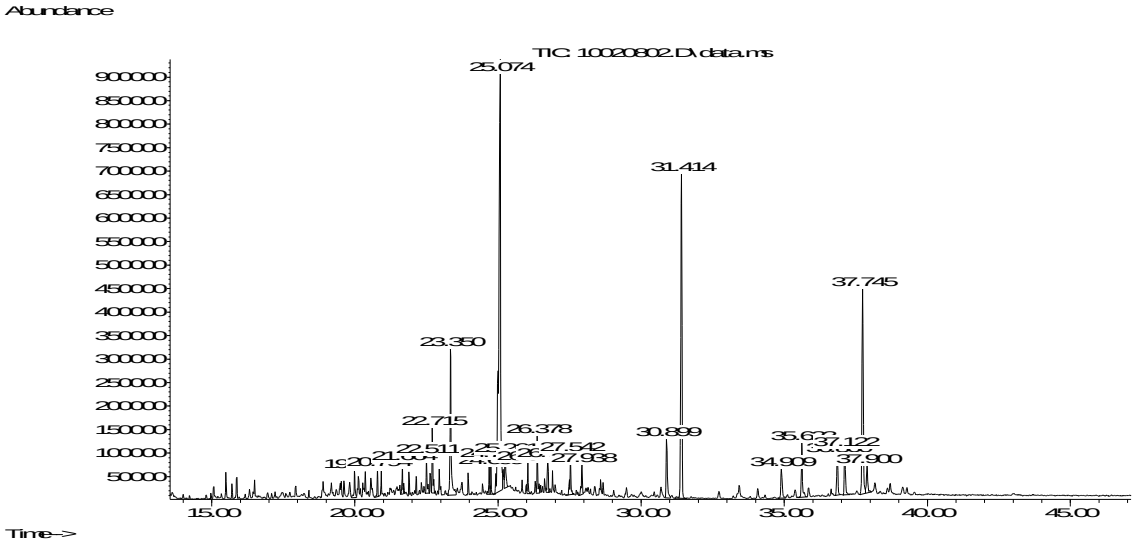
Table A- 2. PAHs identified in the woodsmoke samples

PAH	PM10 Filter samples (ng/mg PM)		PM2.5 Filter samples (ng/mg PM)		XAD samples (Vapor-Phase) (ng/m^3 air)	
	WS10-1	WS10-2	WS-1	WS-2	WS10-1	Background Air
Naphthalene	<65	<32	<130	<42	**1173**	**635**
2-Methylnaphthalene	<65	<32	<130	<42	**163**	**35**
1-Methylnaphthalene	<65	<32	<130	<42	**102**	**20**
Biphenyl	<65	<32	<130	<42	**43**	<8
2,6-Dimethylnaphthalene (coelute)	<65	<32	<130	<42	**31**	**11**
Acenaphthylene	<65	<32	<130	<42	**90**	<8
Acenaphthene	<65	<32	<130	<42	**12**	<8
2,3,5-Trimethylnaphthalene (coelute)	<65	<32	<130	<42	<8	<8
Fluorene	<65	<32	<130	<42	**31**	<8
Phenanthrene	<65	<32	<130	<42	**48**	**19**
Anthracene	<65	<32	<130	<42	**9**	<8
1-Methylphenanthrene	<65	<32	<130	<42	<8	<8
Fluoranthene	<65	<32	<130	<42	**9**	<8
Pyrene	<65	<32	<130	<42	**15**	<8
Benz(a)anthracene	<65	<32	<130	<42	<8	<8
Chrysene+triphenylene	<65	<32	<130	<42	<8	<8
Benzo(b)fluoranthene	<65	**33**	<130	<42	<8	<8
Benzo(k)fluoranthene	<65	<32	<130	<42	<8	<8
Benzo(e)pyrene	<65	<32	<130	<42	<8	<8
Benzo(a)pyrene	<65	**32**	<130	<42	<8	<8
Perylene	<65	<32	<130	<42	<8	<8
Indeno(1,2,3-cd)pyrene	<65	**42**	<130	<42	<8	<8
Dibenz(a,h)anthracene	<65	<32	<130	<42	<8	<8
Benzo(g,h,i)perylene	<65	**41**	<130	<42	<8	<8

Matched background air was collected for identical times as woodsmoke samples. No PAHs were detected in the background air PM filter samples.

Appendix B. Total Ion Chromatograms of Indoor PM Source Samples

Figure B-1. Stir-fry cooking (CK-1) PM10, PMeq injected = 14 µg



Pk# = peak no. RT: retension time. CAS: Chem abstracts registry no. Qual = percent match

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	19.999	1.31	C:\Database\NIST05.L			
			Cyclopentane, 1,2,3-trimethyl-, (1.alpha.,2.alpha.,3.alpha.)-	6658	002613-69-6	59
			Heptanoic acid, 3-methylbutyl este	57118	000109-25-1	56
			Piperazine, 1-[(2,4-dichlorobenzoyl) methyl]-4-methyl-	115638	1000137-95-1	53
2	20.799	0.88	C:\Database\NIST05.L			
			Oxirane, hexadecyl-	104255	007390-81-0	91
			Pentadecanal-	76023	002765-11-9	87
			1,15-Pentadecanediol	88044	014722-40-8	83
3	21.66	0.97	C:\Database\NIST05.L			
			cis-9-Hexadecenal	83993	056219-04-6	94
			13-Octadecenal, (Z)-	102823	058594-45-9	91
			9-Tetradecenal, (Z)-	64377	053939-27-8	91
4	22.506	1.42	C:\Database\NIST05.L			
			Pentadecanenitrile	73708	018300-91-9	47
			Z,Z-6,13-Octadecadien-1-ol acetate	129827	1000131-07-0	38
			3,6-Dimethyl-5-hepten-1-ol acetate	45767	1000131-31-1	30

5	22.722	2.4	C:\Database\NIST05.L			
			1-Pentadecyne	63039	000765-13-9	89
			8-Hexadecenal, 14-methyl-, (Z)-	93524	060609-53-2	89
			13-Octadecenal, (Z)-	102822	058594-45-9	87
6	23.352	8.04	C:\Database\NIST05.L			
			n-Hexadecanoic acid	96235	000057-10-3	96
			n-Hexadecanoic acid	96234	000057-10-3	95
			n-Hexadecanoic acid	96233	000057-10-3	76
7	24.706	0.95	C:\Database\NIST05.L			
			14-Octadecenoic acid, methyl ester	122314	056554-48-4	46
			10-Octadecenoic acid, methyl ester	122312	013481-95-3	46
			Z-8-Methyl-9-tetradecenoic acid	85352	1000130-84-5	38
8	24.752	1.25	C:\Database\NIST05.L			
			Sulfurous acid, isohexyl 2-pentyl ester	82081	1000309-15-5	50
			1,3,2-Oxazaborolane, 2-butyl-	11376	031748-10-4	47
			2(3H)-Furanone, dihydro-5-pentyl-	27819	000104-61-0	47
9	25.075	30.52	C:\Database\NIST05.L			
			Oleic Acid	113354	000112-80-1	99
			6-Octadecenoic acid, (Z)-	113359	000593-39-5	98
			Oleic Acid	113353	000112-80-1	93
10	25.26	1.78	C:\Database\NIST05.L			
			Octadecanoic acid	114821	000057-11-4	89
			Octadecanoic acid	114820	000057-11-4	78
			Octadecanoic acid, 2-(2-hydroxy ethoxy)ethyl ester	162264	000106-11-6	58
11	26.044	1.03	C:\Database\NIST05.L			
			Oxirane, tetradecyl-	85507	007320-37-8	53
			Cyclopentane, 1,1'-[3-(2-cyclopentyl ethyl)-1,5-pentanediyl]bis-	127513	055255-85-1	43
			2-Methyl-E-7-hexadecene	84047	064183-52-4	43
12	26.383	2.15	C:\Database\NIST05.L			
			1-Hexadecyne	73057	000629-74-3	70
			9,12-Octadecadienoic acid (Z,Z)-	111993	000060-33-3	70
			3,4-Octadiene, 7-methyl-	10330	037050-05-8	60
13	26.737	1.33	C:\Database\NIST05.L			
			2-Methyl-Z,Z-3,13-octadecadienol	112083	1000130-90-5	49
			1,6-Octadiene, 5,7-dimethyl-, (R)-	16379	085006-04-8	46
			Z,E-3,13-Octadecadien-1-ol	102833	1000131-10-4	46
14	27.536	1.8	C:\Database\NIST05.L			
			9-Octadecenal, (Z)-	102821	002423-10-1	74

			11-Hexadecynal	82615	086426-73-5	55
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	55
15	27.936	1.36	C:\Database\NIST05.L			
			9,17-Octadecadienal, (Z)-	101505	056554-35-9	40
			2,3-Dihydroxypropyl elaidate	155383	002716-53-2	30
			13-Octadecenal, (Z)-	102823	058594-45-9	30
16	30.905	3.26	C:\Database\NIST05.L			
			Erucic acid	146863	000112-86-7	43
			4-Butyl-1,3-thiazole	18308	053833-33-3	43
			1-Octadecene	93542	000112-88-9	41
17	31.413	17.51	C:\Database\NIST05.L			
			2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	173571	000111-02-4	99
			Squalene	173555	007683-64-9	98
			Squalene	173554	007683-64-9	96
18	34.905	1.77	C:\Database\NIST05.L			
			.gamma.-Tocopherol	174833	007616-22-0	97
			.gamma.-Tocopherol	174832	007616-22-0	93
			Benzenepropanenitrile, 3,4-dimethoxy-	50488	049621-56-9	49
19	35.628	2.94	C:\Database\NIST05.L			
			Heneicosane, 11-decyl-	178194	055320-06-4	91
			Pentatriacontane	184022	000630-07-9	91
			Tetratriacontane	182859	014167-59-0	90
20	36.859	2.22	C:\Database\NIST05.L			
			5-Cholestene-3-ol, 24-methyl-	171443	1000214-17-4	98
			Campesterol	171432	000474-62-4	91
			Ergost-5-en-3-ol, (3.beta.)-	171440	004651-51-8	91
21	37.12	2.44	C:\Database\NIST05.L			
			Stigmasterol	173931	000083-48-7	99
			Stigmasterol	173932	000083-48-7	70
			Chondrillasterol	173934	000481-17-4	49
22	37.751	11.03	C:\Database\NIST05.L			
			.gamma.-Sitosterol	174402	000083-47-6	99
			.beta.-Sitosterol	174399	000083-46-5	96
			.beta.-Sitosterol	174400	000083-46-5	90
23	37.905	1.63	C:\Database\NIST05.L			
			Stigmasta-5,24(28)-dien-3-ol, (3.beta., 24Z)-	173945	000481-14-1	89
			Fucosterol	173929	017605-67-3	64
			5-Androsten-17.alpha.-ethynyl-3.beta., 17.beta.-diol	133597	1000126-90-5	53

Figure B-2. Oven cooking (CK-2) PM10, PMeq injected = 0.1 µg

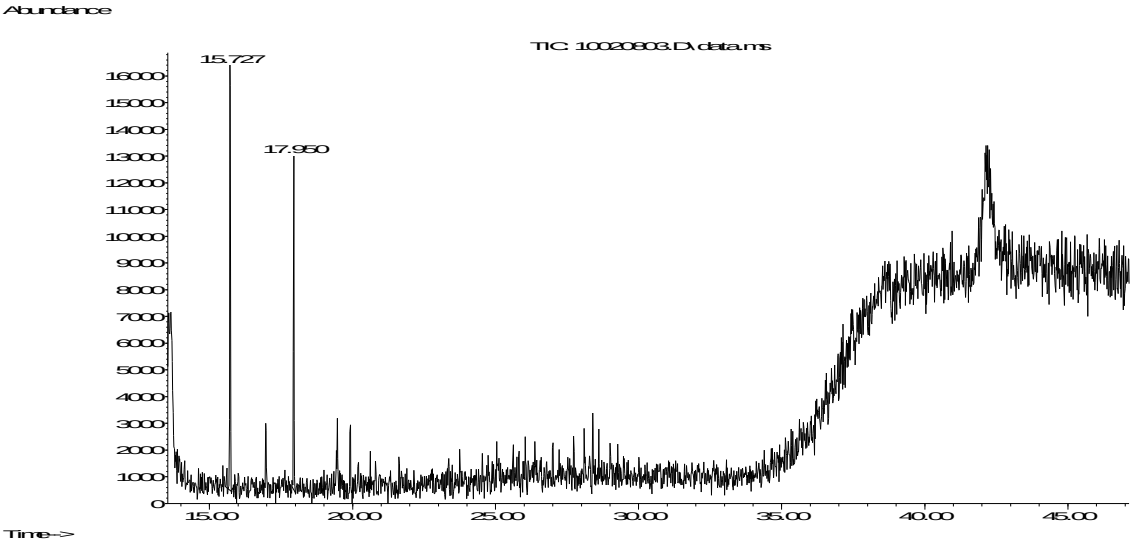
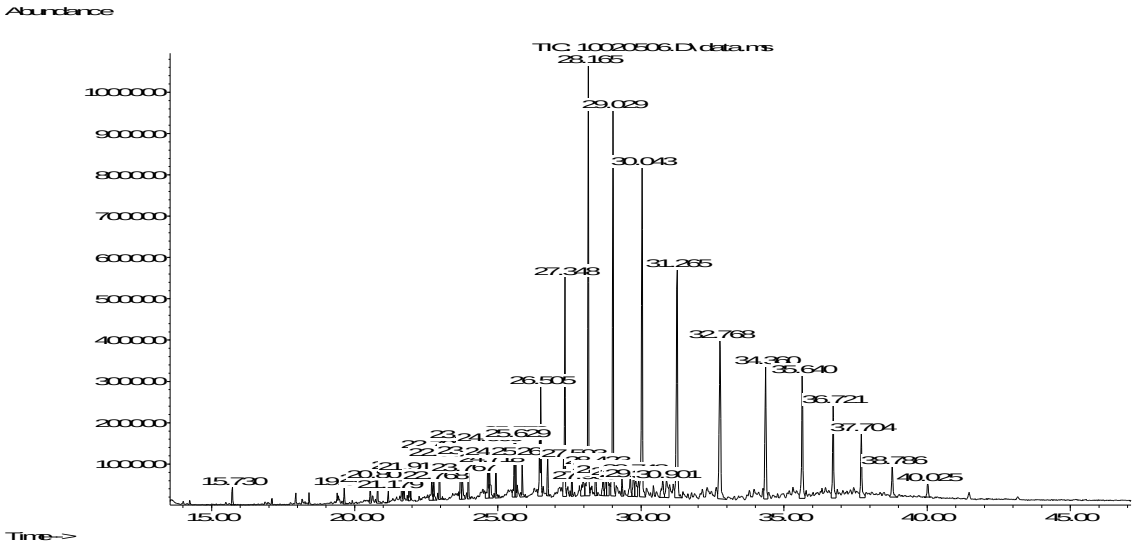


Figure B-3. Candle (CN-C) DCM, PMeq injected = 2.4 µg



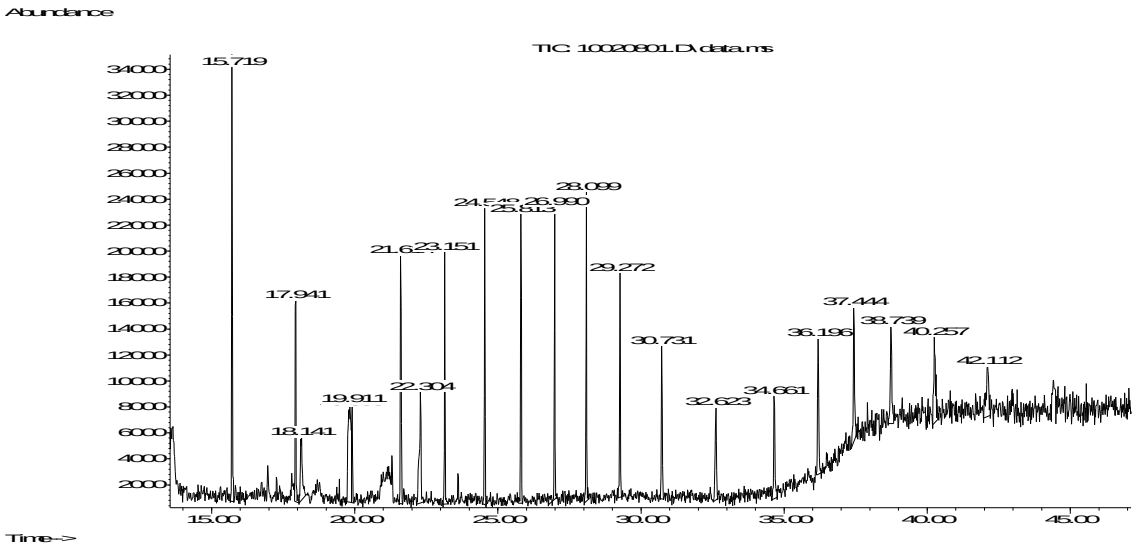
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	21.65	0.75	C:\Database\NIST05.L			
			1-Octadecene	93543	000112-88-9	97
			E-15-Heptadecenal	93518	1000130-97-9	95
			5-Eicosene, (E)-	112105	074685-30-6	91
2	21.911	0.64	C:\Database\NIST05.L			
			Oxirane, hexadecyl-	104255	007390-81-0	91
			1,15-Pentadecanediol	88044	014722-40-8	90
			1,15-Hexadecadiene	73063	021964-51-2	90
3	22.696	1.41	C:\Database\NIST05.L			
			1-Nonadecene	102860	018435-45-5	99
			Pentafluoropropionic acid, heptadecyl ester	171753	1000283-04-2	91
			Carbonic acid, octadecyl 2,2,2-trichloroethyl ester	179187	1000314-56-3	91
4	22.973	1.05	C:\Database\NIST05.L			
			Oxirane, tetradecyl-	85503	007320-37-8	90
			Oxirane, hexadecyl-	104256	007390-81-0	83
			Pentadecanal-	76023	002765-11-9	64
5	23.711	1.62	C:\Database\NIST05.L			
			1-Eicosene	112101	003452-07-1	91
			Heptafluorobutanoic acid, heptadecyl ester	180157	1000282-97-3	90
			Pentafluoropropionic acid, heptadecyl ester	171753	1000283-04-2	90
6	23.973	1	C:\Database\NIST05.L			

			Oxirane, hexadecyl-	104256	007390-81-0	91
			Octadecanal	104241	000638-66-4	91
			1,15-Hexadecadiene	73063	021964-51-2	89
7	24.665	1.35	C:\Database\NIST05.L			
			1-Docosene	129889	001599-67-3	91
			10-Heneicosene (c,t)	121168	095008-11-0	91
			1-Heneicosanol	132419	015594-90-8	91
8	24.711	1.02	C:\Database\NIST05.L			
			Heneicosane	122436	000629-94-7	98
			Octacosane	169720	000630-02-4	90
			Tetratriacontane	182859	014167-59-0	90
9	24.942	1.08	C:\Database\NIST05.L			
			Oxirane, tetradecyl-	85503	007320-37-8	91
			Pentadecanal-	76023	002765-11-9	91
			Oxirane, tridecyl-	76039	018633-25-5	91
10	25.573	1.58	C:\Database\NIST05.L			
			1-Nonadecene	102860	018435-45-5	91
			1-Docosene	129889	001599-67-3	91
			1-Nonadecene	102859	018435-45-5	91
11	25.634	1.44	C:\Database\NIST05.L			
			Docosane	131157	000629-97-0	95
			Tetratriacontane	182859	014167-59-0	90
			Tetratetracontane	188838	007098-22-8	90
12	25.865	1.06	C:\Database\NIST05.L			
			1,19-Eicosadiene	110850	014811-95-1	96
			Z-14-Octadecen-1-ol acetate	131075	1000131-07-6	91
			Oxirane, hexadecyl-	104256	007390-81-0	87
13	26.511	3.77	C:\Database\NIST05.L			
			Hentriacontane	178193	000630-04-6	91
			Heptadecane, 9-octyl-	153748	007225-64-1	91
			Octacosane	169720	000630-02-4	90
14	26.742	0.79	C:\Database\NIST05.L			
			Pentadecanal-	76023	002765-11-9	91
			Oxirane, hexadecyl-	104254	007390-81-0	91
			Octadecanal	104241	000638-66-4	91
15	27.342	6.3	C:\Database\NIST05.L			
			Tetracosane	146923	000646-31-1	99
			Tetracosane	146921	000646-31-1	98
			Tetracosane	146924	000646-31-1	97
16	27.588	0.82	C:\Database\NIST05.L			

			1,21-Docosadiene	128703	053057-53-7	98
			Bicyclo[10.8.0]eicosane, cis-	110854	1000155-82-2	94
			Bicyclo[10.8.0]eicosane, (E)-	110853	1000155-85-0	93
17	28.172	12.49	C:\Database\NIST05.L			
			Docosane	131157	000629-97-0	96
			Hexadecane, 2,6,10,14-tetramethyl-	113503	000638-36-8	92
			Tetracosane, 11-decyl-	182862	055429-84-0	91
18	28.419	0.73	C:\Database\NIST05.L			
			Oxirane, hexadecyl-	104256	007390-81-0	91
			Ethanol, 2-(9-octadecenyloxy)-,	132342	005353-25-3	86
			(Z)-Pentadecanal-	76023	002765-11-9	83
19	29.034	12.12	C:\Database\NIST05.L			
			Hexacosane	159836	000630-01-3	98
			Hexacosane	159837	000630-01-3	97
			Docosane	131157	000629-97-0	97
20	30.049	12.29	C:\Database\NIST05.L			
			Heptacosane	165300	000593-49-7	98
			Docosane	131157	000629-97-0	97
			Hexadecane, 2,6,10,14-tetramethyl-	113507	000638-36-8	96
21	31.264	9.95	C:\Database\NIST05.L			
			Octacosane	169720	000630-02-4	99
			Docosane	131157	000629-97-0	97
			Hexadecane, 2,6,10,14-tetramethyl-	113507	000638-36-8	92
22	32.772	8.37	C:\Database\NIST05.L			
			Nonacosane	173139	000630-03-5	97
			Docosane	131157	000629-97-0	97
			Hexadecane, 2,6,10,14-tetramethyl-	113507	000638-36-8	96
23	34.356	6.18	C:\Database\NIST05.L			
			Tetratriacontane	182859	014167-59-0	94
			Triacontane	175876	000638-68-6	91
			Hexacosane, 9-octyl-	182860	055429-83-9	91
24	35.633	4.93	C:\Database\NIST05.L			
			Hentriacontane	178193	000630-04-6	94
			Tetratriacontane	182859	014167-59-0	91
			Triacontane, 1-bromo-	184514	004209-22-7	91
25	36.725	3.5	C:\Database\NIST05.L			
			Pentatriacontane	184022	000630-07-9	93
			3-Eicosene, (E)-	112107	074685-33-9	92
			E-14-Hexadecenal	83987	330207-53-9	92
26	37.71	2.35	C:\Database\NIST05.L			

			Hexatriacontane	184951	000630-06-8	90
			Sulfurous acid, butyl tetradecyl ester	144698	1000309-18-1	90
			Octacosane	169720	000630-02-4	87
27	38.787	1.43	C:\Database\NIST05.L			
			Pentatriacontane	184022	000630-07-9	87
			Octacosane	169720	000630-02-4	87
			Heneicosane, 11-decyl-	178194	055320-06-4	87

Figure B-4. Woodsmoke (WS10-2) PM10, PMeq injected = 0.27 µg



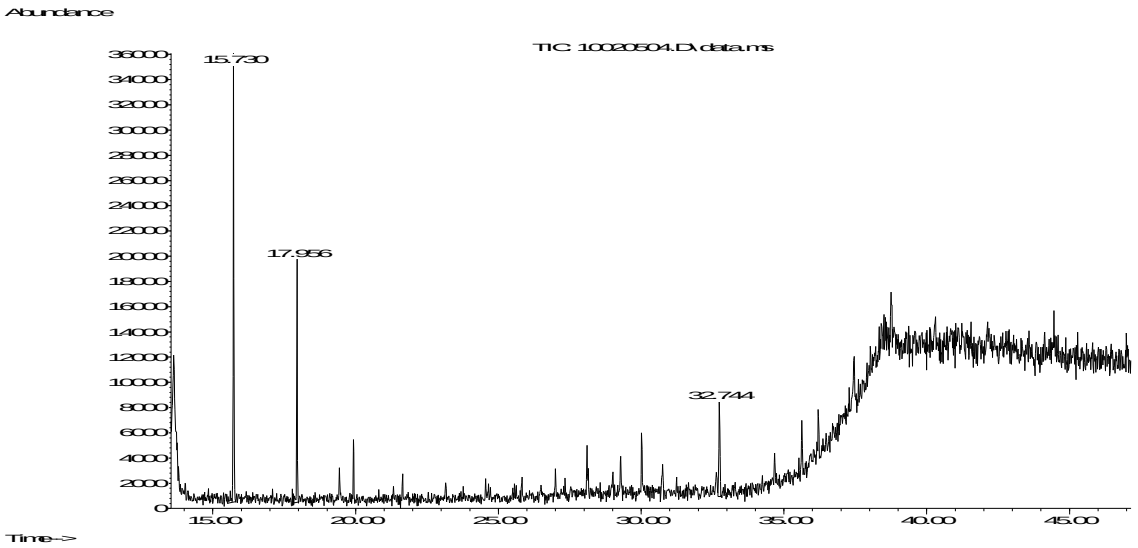
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.711	8.61	C:\Database\NIST05.L			
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	91
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	87
			Acetic acid, [bis[(trimethylsilyl) oxy]phosphinyl]-, trimethylsilyl ester	155042	053044-27-2	40
2	17.942	4.77	C:\Database\NIST05.L			
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	45
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	25
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	25
3	18.142	3.3	C:\Database\NIST05.L			
			3,4-Dihydroxy-5-methyl-dihydrofuran -2-one	13798	1000193-83-1	42
			Xylopyranoside, methyl 4-azido-4- deoxy-, .beta.-L-	49430	020379-31-1	40
			Methyl-4-azido-4-desoxy.beta.l- arabinopyranoside	49429	1000312-10-3	36
4	19.834	7.18	C:\Database\NIST05.L			
			Cyclopentasiloxane, decamethyl-	161016	000541-02-6	32
			Benzoic acid, 2,4-bis[(trimethylsilyl) oxy]-, trimethylsilyl ester	161138	010586-16-0	32
			Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	161132	003618-20-0	23
5	19.911	2.69	C:\Database\NIST05.L			
			N-Methyladrenaline, tri-TMS	174020	1000071-72-1	23
			Cyclopentasiloxane, decamethyl-	161016	000541-02-6	16

			N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrin	187076	1000072-26-7	12
6	21.618	6.4	C:\Database\NIST05.L			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	179156	038147-00-1	45
			Cyclohexasiloxane, dodecamethyl-	179151	000540-97-6	43
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	32
7	22.311	4.9	C:\Database\NIST05.L			
			1,3-Dithiolo[4,5-b][1,3]dithiolo[4,5-E]pyridine-2,6-dione, 8-(trifluoromethyl)-	140869	1000305-32-3	14
			6,8-Difluoro-2,2,4,4,6,7,7,8,9,9-decamethyl-[1,3,5,2,4,6,7,8,9]trioxahexasilonane	172136	1000311-72-2	12
			4-[4-[p-[n-Hexyloxyphenyl]butylamino]-1,2-naphthoquinone	172544	025107-58-8	10
8	23.157	4.61	C:\Database\NIST05.L			
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	184742	019095-23-9	35
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	17
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	14
9	24.541	5.03	C:\Database\NIST05.L			
			N-Methyladrenaline, tri-TMS	174020	1000071-72-1	25
			Silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl)oxy]phenyl]-1,1,1-trimethyl-	112442	072088-09-6	10
			N-Benzyl-N-ethyl-p-isopropylbenzamide	112663	015089-22-2	10
10	25.818	5.67	C:\Database\NIST05.L			
			Phenethylamine, N-methyl-.beta.,3,4-tris(trimethylsiloxy)-	170947	010538-85-9	22
			1,3,5,7,9-Pentaethylbicyclo[5.3.1]pentasiloxane	166183	073420-26-5	22
			Norcodeine di-TMS derivative	177056	1000137-11-3	14
11	26.987	5.65	C:\Database\NIST05.L			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	179156	038147-00-1	42
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	184742	019095-23-9	37
			Dithioerythritol, O,O',S,S'-tetrakis(trimethylsilyl)-	178865	1000079-30-7	22
12	28.095	5.73	C:\Database\NIST05.L			
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	38
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	184742	019095-23-9	37

			N-Benzyl-N-ethyl-p-isopropylbenzamide	112663	015089-22-2	35
13	29.279	4.74	C:\Database\NIST05.L			
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	23
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	16
			N-Methyladrenaline, tri-TMS	174020	1000071-72-1	10
14	30.725	3.83	C:\Database\NIST05.L			
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	50
			3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-	120498	004342-25-0	32
			2-(2,6,6-Trimethylcyclohex-1-enyl) cyclopropanecarboxylic acid, methyl ester	72797	1000185-64-1	27
15	32.617	3.13	C:\Database\NIST05.L			
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	47
			3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-	120498	004342-25-0	22
			2-(2,6,6-Trimethylcyclohex-1-enyl) cyclopropanecarboxylic acid, methyl ester	72797	1000185-64-1	14
16	34.663	3.56	C:\Database\NIST05.L			
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	40
			1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane	166197	038146-99-5	35
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	32
17	36.202	4.03	C:\Database\NIST05.L			
			1-Monolinoleoylglycerol trimethylsilyl ether	184354	054284-45-6	37
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	25
			Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-	72681	055012-80-1	14
18	37.448	4.41	C:\Database\NIST05.L			
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-	184742	019095-23-9	37
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	35
			Pentasiloxane, dodecamethyl-	166196	000141-63-9	27
19	38.74	3.96	C:\Database\NIST05.L			
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	53
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-	184742	019095-23-9	40
			1-Monolinoleoylglycerol trimethylsilyl	184354	054284-45-6	38

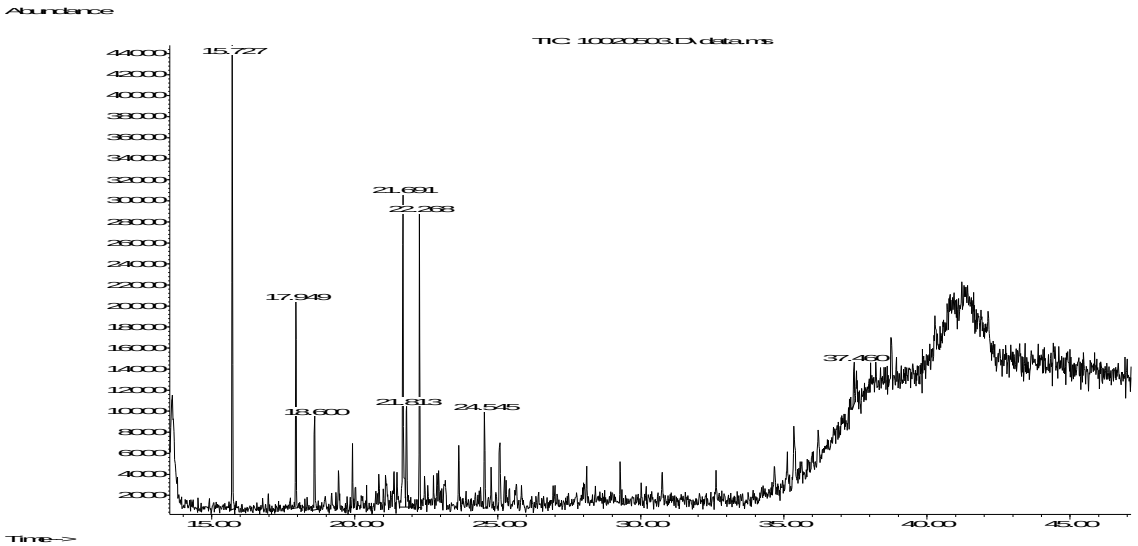
			ether			
20	40.263	4.18	C:\Database\NIST05.L			
			1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	154967	1000316-17-5	43
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	184742	019095-23-9	42
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	38
21	42.109	3.6	C:\Database\NIST05.L			
			Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	62024	1000129-52-1	43
			Cyclotrisiloxane, hexamethyl-	73121	000541-05-9	38
			Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-	101373	330455-64-6	38

Figure B-5. Incense (INC-Ga, Floral India) PM2.5 F1, PMeq injected = 37 µg



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.737	54.76	C:\Database\NIST05.L			
			Acetic acid, [bis[(trimethylsilyl) oxy]phosphinyl]-, trimethylsilyl ester	155042	053044-27-2	38
			1,3,5,7,9-Pentaethylcyclopentasiloxane	161018	017995-44-7	32
			3-(6-Methyl-3-pyridyl)-1,5-di(p-tolyl)-2-pyrazoline	148416	010040-66-1	9
2	17.952	28.19	C:\Database\NIST05.L			
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	43
			2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	140587	1000275-63-1	38
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	27
3	32.751	17.05	C:\Database\NIST05.L			
			Tetratetracontane	188837	007098-22-8	59
			1-Iodo-2-methylnonane	103530	1000101-47-9	50
			Disulfide, di-tert-dodecyl	171863	027458-90-8	45

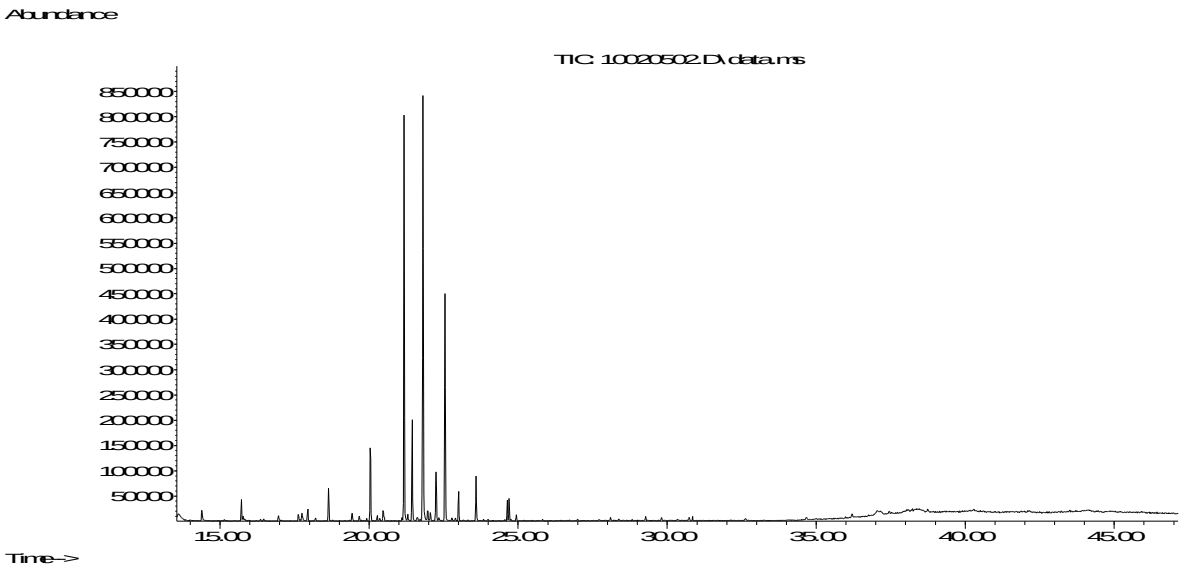
Figure B-6. Incense (INC-Ga, Floral India) PM2.5 F2, PMeq injected = 54 µg



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.722	25.17	C:\Database\NIST05.L			
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	83
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	49
			Acetic acid, [bis[(trimethylsilyl)oxy] phosphinyl]-, trimethylsilyl ester	155042	053044-27-2	42
2	17.952	12.4	C:\Database\NIST05.L			
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	42
			3-Isopropoxy-1,1,1,7,7,7-hexamethy l-3,5,5-tris(trimethylsiloxy)tetra siloxane	187800	071579-69-6	32
			3-Ethoxy-1,1,1,5,5,5-hexamethyl-3- (trimethylsiloxy)trisiloxane	147384	018030-67-6	16
3	18.598	6.7	C:\Database\NIST05.L			
			Dibenzofuran	35097	000132-64-9	59
			3,5-Dimethoxybenzyl alcohol	35492	000705-76-0	50
			Pyrimidine, 2-(dimethylamino)-5-nitro	35249	014233-44-4	50
4	21.69	23.54	C:\Database\NIST05.L			
			Phenanthrene	41767	000085-01-8	93
			Anthracene	41762	000120-12-7	91
			Anthracene	41759	000120-12-7	87
5	21.813	8.07	C:\Database\NIST05.L			
			Anthracene	41759	000120-12-7	43
			Phenanthrene	41763	000085-01-8	43
			Phenanthrene	41767	000085-01-8	43

6	22.275	14.85	C:\Database\NIST05.L			
			Benzene, 1-(1,1-dimethylethyl)-3,5-dimethyl-2,4,6-trinitro-	122559	000081-15-2	94
			Benzene, 1-(1,1-dimethylethyl)-3,5-dimethyl-2,4,6-trinitro-	122560	000081-15-2	87
			7-Methoxy-3-(p-methoxyphenyl)-4H-chromen-4-one	113214	001157-39-7	52
7	24.552	6.46	C:\Database\NIST05.L			
			1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro-	58195	1000302-68-2	9
			4,5-Dihydronaphtho(2,1-d)thiazol-2 amine	58202	034176-49-3	9
			9H-1,2,4,9-Tetraazafluorene-3-thio	58794	1000303-64-3	9
8	37.458	2.82	C:\Database\NIST05.L			
			Silicic acid, diethyl bis(trimethylsilyl) ester	121708	003555-45-1	38
			1,2-Bis(trimethylsilyl)benzene	72520	017151-09-6	38
			Silane, 1,4-phenylenebis[trimethyl	72522	013183-70-5	38

Figure B-7. Incense (INC-Ga, Floral India) PM2.5 F3, PMeq injected = 45 µg

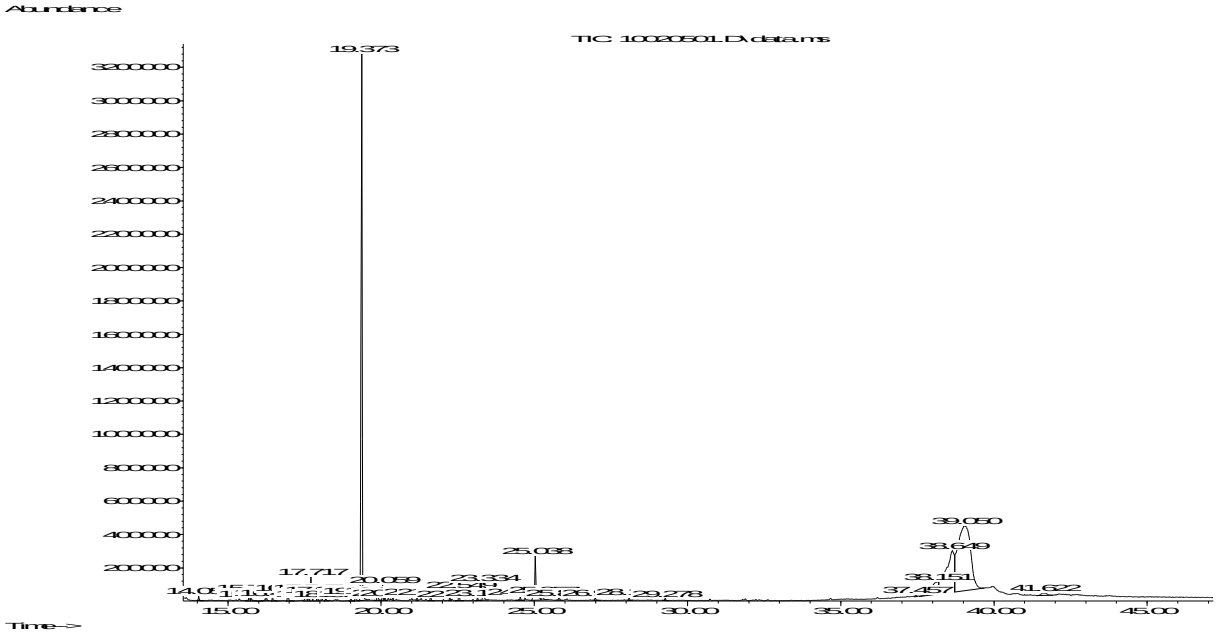


Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	14.39	1.03	C:\Database\NIST05.L			
			Benzofuran, 2,3-dihydro-	9098	000496-16-2	64
			N-Benzyl-2-phenethylamine	65153	003647-71-0	53
			Catecholborane	9047	000274-07-7	50
2	15.729	1.37	C:\Database\NIST05.L			
			Acetic acid, [bis[(trimethylsilyl) oxy]phosphinyl]-, trimethylsilyl ester	155042	053044-27-2	38
			1,3,5,7,9-Pentaethylcyclopenta siloxane	161018	017995-44-7	25
			3-Demethyl-3-ethylthiocolchicine	177046	097043-00-0	14
3	17.759	0.8	C:\Database\NIST05.L			
			2-Cyclohexen-1-ol	3119	000822-67-3	37
			1,2-Cyclohexanediol	7861	000931-17-9	33
			1,2-Cyclohexanediol	7865	000931-17-9	33
4	17.944	0.77	C:\Database\NIST05.L			
			2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	140587	1000275-63-1	43
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	43
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	33

5	18.651	2.09	C:\Database\NIST05.L			
			Lilial (Methyl, beta-(p-tert-butylphenyl)propionaldehyde)	59722	000080-54-6	98
			Lilial	59721	000080-54-6	97
			Benzene, 1,3,5-tris(1-methylethyl)	59875	000717-74-8	64
6	19.436	0.62	C:\Database\NIST05.L			
			Diphenyl sulfide	47396	000139-66-2	87
			Diphenyl sulfide	47398	000139-66-2	80
			1-Naphthalenecarboxaldehyde, 4-methoxy-	47385	015971-29-6	72
7	20.051	4.95	C:\Database\NIST05.L			
			Heptanal, 2-(phenylmethylene)-	58469	000122-40-7	90
			Heptanal, 2-(phenylmethylene)-	58467	000122-40-7	50
			1-(2-Methyl-allyl)-1,2,3,4-tetrahydro naphthalen-2-ol	58495	1000192-52-9	47
8	20.482	0.96	C:\Database\NIST05.L			
			Phenol, 2-(phenylmethyl)-	46042	028994-41-4	94
			Phenol, 2-(phenylmethyl)-	46055	028994-41-4	81
			Phenol, 4-(phenylmethyl)-	46046	000101-53-1	58
9	21.174	23.89	C:\Database\NIST05.L			
			Octanal, 2-(phenylmethylene)-	68638	000101-86-0	99
			Octanal, 2-(phenylmethylene)-	68636	000101-86-0	99
			Octanal, 2-(phenylmethylene)-	68637	000101-86-0	94
10	21.451	6.74	C:\Database\NIST05.L			
			Benzyl Benzoate	65860	000120-51-4	95
			Benzyl Benzoate	65863	000120-51-4	93
			Octanal, 2-(phenylmethylene)-	68636	000101-86-0	90
11	21.82	29.82	C:\Database\NIST05.L			
			1-Naphthalenol, 5,6,7,8-tetrahydro -2,5-dimethyl-8-(1-methylethyl)-	69965	055012-72-1	80
			Benzene, 1,4-bis(1,1-dimethylethyl)-	50015	001012-72-2	72
			2H-1-Benzopyran, 7-methoxy-2,2-dimethyl-	49846	017598-02-6	64
12	21.974	0.83	C:\Database\NIST05.L			
			2H-Isoindole, 4,5,6,7-tetramethyl-	38542	070187-61-0	50
			1,3,5-Triazine-2,4-diamine, 6-chloro -N-ethyl-	38599	001007-28-9	38
			1,4-Naphthalenedione, 2-acetyl-3-hydroxy-	68428	002246-48-2	38
13	22.066	0.65	C:\Database\NIST05.L			
			2,3,4,5-Tetrahydro-8-methoxy-2-	68527	041505-84-4	58

			methyl-1H-pyrido[4,3-b]indole			
			Benzyl alcohol, .alpha.-isobutyl-2, 4,5-trimethyl-	61463	010425-87-3	53
			1H-Inden-1-one, 2,3-dihydro-3,3,5, 6-tetramethyl-	48732	054789-22-9	50
14	22.251	4	C:\Database\NIST05.L			
			7-Acetyl-6-ethyl-1,1,4,4-tetramethyl tetralin	97610	000088-29-9	97
			Cyclopenta[g]-2-benzopyran, 1,3,4, 6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	97614	001222-05-5	96
			Galaxolide 1 (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran) musk, floral, woody odor fragrance	97603	1000285-26-6	94
15	22.559	14.45	C:\Database\NIST05.L			
			Benzoic acid, 2-hydroxy-, phenylmethyl ester	77199	000118-58-1	95
			Benzoic acid, 2-hydroxy-, phenylmethyl ester	77200	000118-58-1	93
			Benzoic acid, 2-hydroxy-, phenylmethyl ester	77201	000118-58-1	90
16	23.005	1.7	C:\Database\NIST05.L			
			Pentadecanoic acid, 14-methyl-, methyl ester	105659	005129-60-2	97
			Hexadecanoic acid, methyl ester	105645	000112-39-0	96
			Hexadecanoic acid, methyl ester	105639	000112-39-0	95
17	23.589	2.9	C:\Database\NIST05.L			
			7-Hydroxycadalene	67344	002102-75-2	95
			Naphthalene, 1-(1,1-dimethylethyl) -7-methoxy-	67353	060683-42-3	91
			Ethanone, 1-(4'-fluoro[1,1'-biphenyl] -4-yl)-	67230	000720-74-1	72
18	24.635	1.08	C:\Database\NIST05.L			
			9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	121114	017309-05-6	98
			9,12-Octadecadienoic acid, methyl ester	121093	002462-85-3	96
			9,12-Octadecadienoic acid, methyl ester, (E,E)-	121109	002566-97-4	83
19	24.712	1.36	C:\Database\NIST05.L			
			14-Octadecenoic acid, methyl ester	122314	056554-48-4	50
			10-Octadecenoic acid, methyl ester	122312	013481-95-3	47
			9-Octadecenoic acid (Z)-, methyl ester	122323	000112-62-9	47

Figure B-8. Incense (INC-Ga, Floral India) PM2.5 F4, PMeq injected = 37 µg



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	14.05	0.17	C:\Database\NIST05.L			
			Benzoic acid, 2-hydroxy-, methyl ester	24827	000119-36-8	95
			Benzoic acid, 2-hydroxy-, methyl ester	24832	000119-36-8	93
			Benzoic acid, 2-hydroxy-, methyl ester	24831	000119-36-8	93
2	15.696	0.33	C:\Database\NIST05.L			
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	91
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	87
			Cyclohexasiloxane, dodecamethyl-	179151	000540-97-6	47
3	15.757	0.23	C:\Database\NIST05.L			
			2-Propen-1-ol, 3-phenyl-	14809	000104-54-1	91
			2-Propen-1-ol, 3-phenyl-	14811	000104-54-1	70
			2-Propen-1-ol, 3-phenyl-	14804	000104-54-1	64
4	16.265	0.25	C:\Database\NIST05.L			
			Phenol, 2,6-dimethoxy-	26272	000091-10-1	94
			Phenol, 2,6-dimethoxy-	26275	000091-10-1	93
			Phenol, 3,4-dimethoxy-	26273	002033-89-8	74
5	16.465	0.15	C:\Database\NIST05.L			

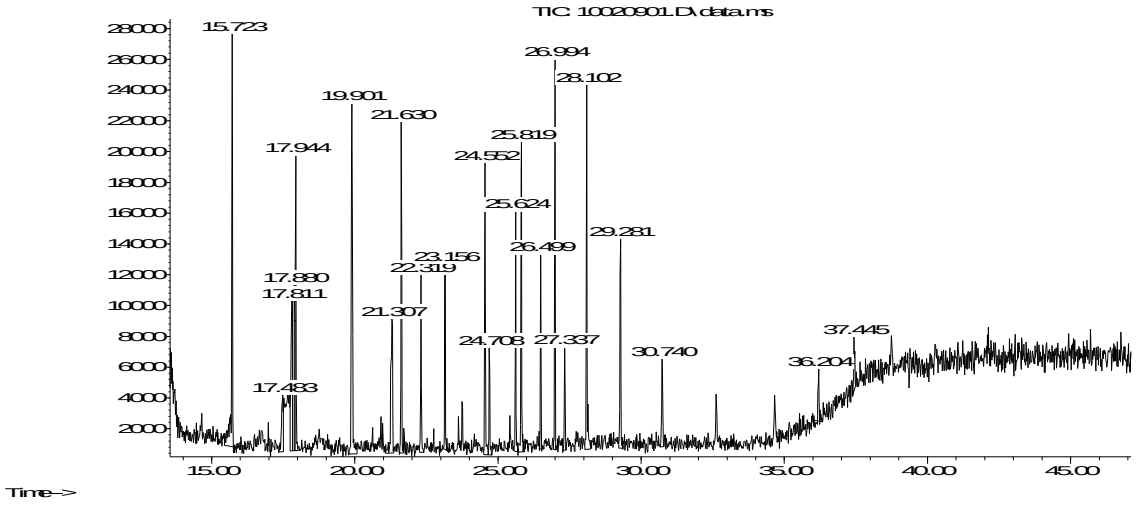
			2(3H)-Furanone, dihydro-5-pentyl-	27819	000104-61-0	72
			2(3H)-Furanone, dihydro-5-pentyl-	27812	000104-61-0	72
			2(3H)-Furanone, dihydro-5-pentyl-	27818	000104-61-0	64
6	16.957	0.34	C:\Database\NIST05.L			
			Vanillin	24743	000121-33-5	98
			Vanillin	24745	000121-33-5	97
			Vanillin	24742	000121-33-5	96
7	17.542	0.48	C:\Database\NIST05.L			
			2H-1-Benzopyran-2-one	21396	000091-64-5	93
			2H-1-Benzopyran-2-one	21395	000091-64-5	89
			2H-1-Benzopyran-2-one	21397	000091-64-5	76
8	17.634	0.16	C:\Database\NIST05.L			
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl -3,5,5-tris(trimethylsiloxy) tetra siloxane	187800	071579-69-6	32
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	27
			Pentasiloxane, dodecamethyl-	166196	000141-63-9	27
9	17.711	1	C:\Database\NIST05.L			
			Ethyl Vanillin	33932	000121-32-4	97
			Ethyl Vanillin	33930	000121-32-4	97
			Ethyl Vanillin	33933	000121-32-4	96
10	17.865	0.45	C:\Database\NIST05.L			
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	16
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl -3,5,5-tris(trimethylsiloxy)tetra siloxane	187800	071579-69-6	12
			Cyclobutene-3,4-dione, 1-dimethyl amino-2-hydroxy-	18239	182881-06-7	10
11	17.926	0.28	C:\Database\NIST05.L			
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	47
			Pentasiloxane, dodecamethyl-	166196	000141-63-9	47
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	43
12	18.203	0.1	C:\Database\NIST05.L			
			Benzene, 1,2-dimethoxy-4-(1-propenyl)	41489	000093-16-3	50
			Benzene, 1,2-dimethoxy-4-(1-propenyl)	41491	000093-16-3	43
			Benzene, 1,2-dimethoxy-4-(1-propenyl)	41485	000093-16-3	38
13	19.019	0.13	C:\Database\NIST05.L			
			2,6,10-Dodecatrien-1-ol, 3,7,11- trimethyl-	72934	004602-84-0	38
			Nerolidol 2	72901	1000285-43-6	38
			Furan, 2,3-dihydro-2,2-dimethyl-3-	43127	077822-49-2	38

			(1-methylethenyl)-5-(1-methylethyl)-			
14	19.172	0.26	C:\Database\NIST05.L			
			2(3H)-Furanone, 5-hexyldihydro-	36134	000706-14-9	72
			2(3H)-Furanone, 5-heptyldihydro-	45756	000104-67-6	72
			2(3H)-Furanone, 5-heptyldihydro-	45752	000104-67-6	64
15	19.372	28.38	C:\Database\NIST05.L			
			Diethyl Phthalate	72414	000084-66-2	97
			Diethyl Phthalate	72415	000084-66-2	90
			Diethyl Phthalate	72412	000084-66-2	90
16	19.895	0.32	C:\Database\NIST05.L			
			Benzoic acid, 2,4-bis[(trimethylsilyl) oxy]-, trimethylsilyl ester	161136	010586-16-0	37
			N-(Trifluoracetyl)-O,O',O''-tris (trimethylsilyl)epinephrine	184164	054135-51-2	35
			Benzoic acid, 2,4-bis[(trimethylsi oxy]-, trimethylsilyl ester	161138	010586-16-0	32
17	20.065	0.6	C:\Database\NIST05.L			
			Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	75715	024851-98-7	74
			Isocitronellol	27048	018479-52-2	47
			Cyclopentane, 1-ethyl-1-methyl-	6619	016747-50-5	35
18	20.126	0.15	C:\Database\NIST05.L			
			Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	45077	000134-96-3	70
			Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	45075	000134-96-3	64
			Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	45076	000134-96-3	58
19	20.388	0.13	C:\Database\NIST05.L			
			Phthalic acid, cyclohexyl phenyl ester	139078	1000315-60-1	47
			Phthalic acid, cyclohexylmethyl ethyl ester	118497	1000309-10-0	47
			Phthalic acid, 2-cyclohexylethyl ethyl ester	127261	1000309-05-4	47
20	21.172	0.17	C:\Database\NIST05.L			
			Octanal, 2-(phenylmethylene)-	68638	000101-86-0	92
			Octanal, 2-(phenylmethylene)-	68634	000101-86-0	68
			Octanal, 2-(phenylmethylene)-	68637	000101-86-0	44
21	21.618	0.17	C:\Database\NIST05.L			
			Mercaptoacetic acid, bis(trimethyl	82769	006398-62-5	25

			silyl)-			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	16
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetra siloxane	187800	071579-69-6	16
22	22.249	0.12	C:\Database\NIST05.L			
			Galaxolide 1	97603	1000285-26-6	90
			Galaxolide 2	97604	1000285-26-7	83
			1-(4-Aminophenyl)-3,6-diazahomoadamantane	87377	148988-05-0	50
23	22.557	0.43	C:\Database\NIST05.L			
			Benzoic acid, 2-hydroxy-, phenylmethyl ester	77199	000118-58-1	87
			4-Benzyloxybenzoic acid	77151	001486-51-7	81
			Benzaldehyde, 3-hydroxy-4-benzyloxy-	77180	004049-39-2	81
24	23.157	0.11	C:\Database\NIST05.L			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	58
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	43
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-	184742	019095-23-9	35
25	23.341	0.9	C:\Database\NIST05.L			
			n-Hexadecanoic acid	96234	000057-10-3	94
			n-Hexadecanoic acid	96233	000057-10-3	90
			n-Hexadecanoic acid	96235	000057-10-3	87
26	24.557	0.15	C:\Database\NIST05.L			
			2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(3-methylbutyl)-1,3-bis (trimethylsilyl)-	161170	052937-67-4	38
			Cyclopentasiloxane, decamethyl-	161015	000541-02-6	25
			1,3,5,7-Tetraethyl-1-ethylbutoxy siloxycyclotetrasiloxane	178859	073420-30-1	17
27	25.033	3.16	C:\Database\NIST05.L			
			Oleic Acid	113354	000112-80-1	92
			Oleic Acid	113353	000112-80-1	91
			6-Octadecenoic acid, (Z)-	113359	000593-39-5	87
28	25.264	0.34	C:\Database\NIST05.L			
			Octadecanoic acid	114822	000057-11-4	64
			Octadecanoic acid	114818	000057-11-4	55
			Octadecanoic acid	114820	000057-11-4	55

29	25.818	0.13	C:\Database\NIST05.L			
			Silane, [[4-[1,2-bis[(trimethylsilyl) oxy]ethyl]-1,2-phenylene]bis(oxy)] bis[trimethyl-	180821	056114-62-6	43
			Silane, [[4-[1,2-bis[(trimethylsilyl) oxy]ethyl]-1,2-phenylene]bis(oxy)] bis[trimethyl-	180822	056114-62-6	43
			Silane, [[4-[1,2-bis[(trimethylsilyl) oxy]ethyl]-1,2-phenylene]bis(oxy)] bis[trimethyl-	180820	056114-62-6	38
30	26.987	0.16	C:\Database\NIST05.L			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	53
			3-Trimethylsilyloxystearic acid, trimethylsilyl ester	179226	1000079-42-6	27
			Mercaptoacetic acid, bis(trimethyl silyl)-	82769	006398-62-5	25
31	28.095	0.12	C:\Database\NIST05.L			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	37
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl -3,5,5-tris(trimethylsiloxy)tetra siloxane	187800	071579-69-6	25
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-	184742	019095-23-9	17
32	29.279	0.12	C:\Database\NIST05.L			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	37
			Carbamic acid, N-(2,3-dimethylphenyl)- oxiranylmethyl ester	71885	339273-79-9	35
			3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-	120498	004342-25-0	35
33	37.463	0.13	C:\Database\NIST05.L			
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	42
			Silicic acid, diethyl bis(trimethlsilyl) ester	121708	003555-45-1	38
			Silane, 1,4-phenylenebis[trimethyl	72522	013183-70-5	35
34	38.155	4.17	C:\Database\NIST05.L			
			2-(Pyridyl)-4,6-bis(4-aminophenyl) pyrimidine	147274	1000078-62-7	30
			Spiro[2,5-cyclohexadiene-1,7'(1'H) -cyclopent[ij]isoquinolin]-4-one,	147251	004880-87-9	30

Figure B-9. Incense (INC-Fa, Mainichi-koh) PM2.5 F1, PMeq injected = 2.3 µg



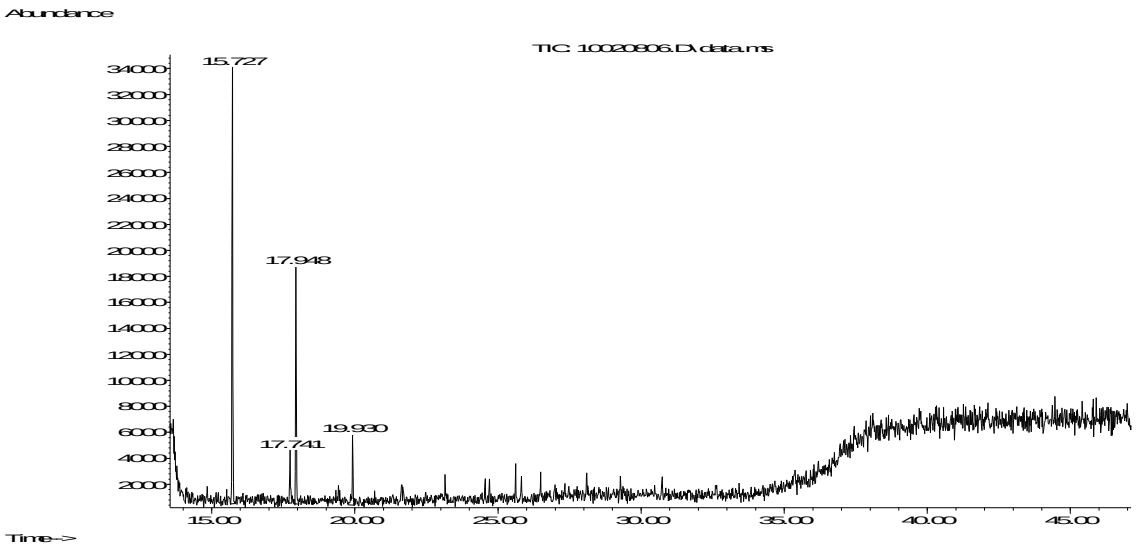
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.728	8.95	C:\Database\NIST05.L			
			Benzenamine, 4-bromo-3-chloro-N-(4-methylthiobenzylydene)-	147003	314283-74-4	43
			1,3,5,7,9-Pentaethylcyclopentasiloxane	161018	017995-44-7	43
			Acetic acid, [bis[(trimethylsilyl)oxy] phosphinyl]-, trimethylsilyl ester	155042	053044-27-2	37
2	17.481	1.99	C:\Database\NIST05.L			
			3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	147384	018030-67-6	25
			Piperidine, 1-(2-chloro-4-nitrobenzoyl)-4-methyl-	112876	282104-35-2	9
			2-Oxo-4-phenyl-6-(4-chlorophenyl)-1,2-dihydropyrimidine	113106	024030-13-5	9
3	17.804	5.54	C:\Database\NIST05.L			
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	47
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	177117	000995-82-4	38
			Pentasiloxane, dodecamethyl-	166196	000141-63-9	37
4	17.881	6.47	C:\Database\NIST05.L			
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	53
			2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	140587	1000275-63-1	43
			3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	147384	018030-67-6	43
5	17.943	6.49	C:\Database\NIST05.L			

			Trisiloxane, 1,1,1,5,5,5-hexamethy l-3,3-bis[(trimethylsilyl)oxy]-	166198	003555-47-3	38
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	38
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	17
6	19.896	10.43	C:\Database\NIST05.L			
			Silane, [[4-[1,2-bis[(trimethylsil yl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	180820	056114-62-6	53
			1,3,5,7,9-Pentaethylbicyclo[5.3.1] pentasiloxane	166183	073420-26-5	43
			1,3,5,7-Tetraethyl-1-ethylbutoxy siloxycyclotetrasiloxane	178859	073420-30-1	43
7	21.312	5.45	C:\Database\NIST05.L			
			2-[2-Thienyl]-4-acetyl quinoline	94002	027302-83-6	28
			3-[p-Methoxyphenyl]-5-methylrhodanine	93745	016711-84-5	9
			1H-1,2,4-Triazole-5(4H)-thione, 4- phenyl-3-(3-pyridyl)-	94374	057600-03-0	9
8	21.635	5.45	C:\Database\NIST05.L			
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	42
			Morphinan, 7,8-didehydro-4,5-epoxy -17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5.alpha.,6.alpha.)-	177057	055449-66-6	38
			Cobalt[ii] bis(O,O'-diethyldithiophosphate)	177109	037511-99-2	25
9	22.312	3	C:\Database\NIST05.L			
			1,3-Dithiolo[4,5-b][1,3]dithiolo[4 ,5-E]pyridine-2,6-dione, 8-(trifluoromethyl)-	140869	1000305-32-3	14
			7-Methoxy-2,3-diphenyl-4H-chromen- 4-one	141495	018720-69-9	12
			4-[4-[p-[n-Hexyloxyphenyl]butylamino] -1,2-naphthoquinone	172544	025107-58-8	12
10	23.158	3.65	C:\Database\NIST05.L			
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	27
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	23
			2-(2',4',4',6',6',8',8'-Heptamethy ltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopenta siloxane	189407	145344-72-5	22
11	24.558	4.97	C:\Database\NIST05.L			
			Benzeneacetic acid, .alpha.,3,4-tris [(trimethylsilyl)oxy]-, trimethylsilyl ester	182236	037148-65-5	50
			n-Nonadecanoic acid, pentamethyldisilyl ester	176847	1000217-02-3	47
			N,N-Dimethyl-N'-(10-propyl-10H-acridin- 9-ylidene)-benzene-1,4-diamin	155019	1000286-20-4	47

12	24.711	2.06	C:\Database\NIST05.L			
			Oxalic acid, 6-ethyloct-3-yl propyl ester	106740	1000309-34-0	64
			Tetratetracontane	188836	007098-22-8	64
			Sulfurous acid, butyl dodecyl este	128314	1000309-17-9	59
13	25.619	3.63	C:\Database\NIST05.L			
			Heptadecane	85525	000629-78-7	80
			Heneicosane	122436	000629-94-7	80
			Heptadecane	85524	000629-78-7	78
14	25.819	6.3	C:\Database\NIST05.L			
			Silane, [[4-[1,2-bis[(trimethylsilyl)oxy)] ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	180820	056114-62-6	27
			Pyrazolo[3,4-b]pyridin-3(2H)-one, 4-trifluoromethyl-2,6-diphenyl-	154853	309740-17-8	10
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	10
15	26.496	3.06	C:\Database\NIST05.L			
			Octacosane	169720	000630-02-4	72
			Tetratriacontane	182859	014167-59-0	72
			Heptacosane	165300	000593-49-7	64
16	26.988	5.6	C:\Database\NIST05.L			
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	187862	019095-24-0	32
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	28
			N-Benzyl-N-ethyl-p-isopropylbenzamide	112663	015089-22-2	27
17	27.342	1.78	C:\Database\NIST05.L			
			Dodecane, 1-iodo-	121770	004292-19-7	36
			1-Hexanol, 5-methyl-2-(1-methylethyl)-	28408	002051-33-4	36
			Nonane, 1-iodo-	95058	004282-42-2	33
18	28.096	6.2	C:\Database\NIST05.L			
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetra siloxane	187800	071579-69-6	32
			N-Benzyl-N-ethyl-p-isopropylbenzamide	112663	015089-22-2	30
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	22
19	29.28	4.35	C:\Database\NIST05.L			
			3-Isopropoxy-1,1,1,7,7,7-hexamethy l-3,5,5-tris(trimethylsiloxy)tetrasiloxane	187800	071579-69-6	43
			1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy)tetrasiloxane	179156	038147-00-1	28
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	27

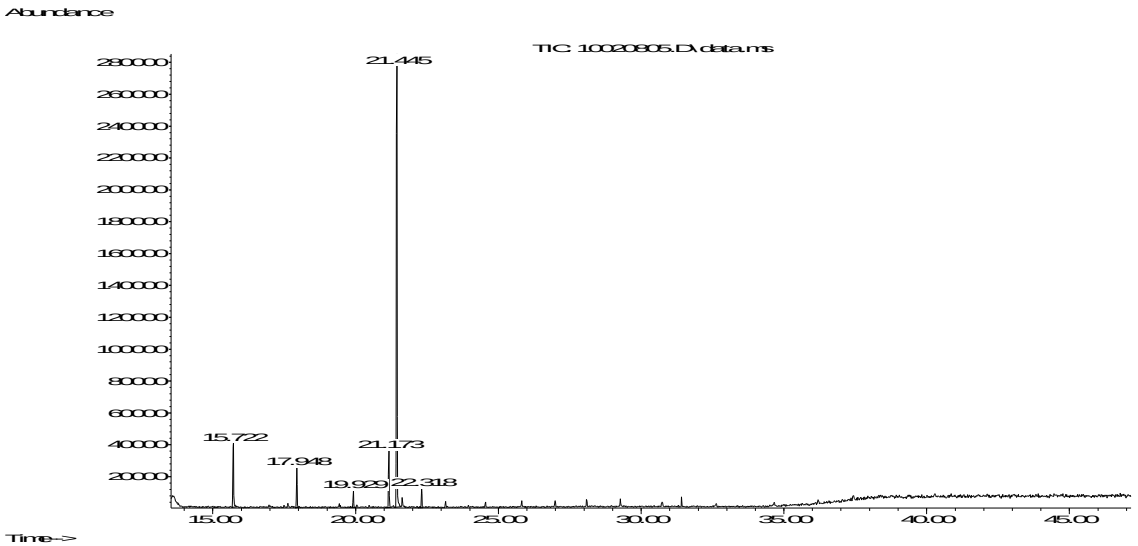
20	30.742	2.25	C:\Database\NIST05.L			
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	37
			9,12,15-Octadecatrienoic acid, 2,3 -bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	184243	055521-22-7	10
			Trisiloxane, 1,1,1,5,5,5-hexamethy l-3,3-bis[(trimethylsilyl)oxy]-	166198	003555-47-3	10
21	36.203	1.14	C:\Database\NIST05.L			
			2-Ethylacridine	62222	055751-83-2	38
			Thiocarbamic acid, N,N-dimethyl, S -1,3-diphenyl-2-butenyl ester	131579	1000192-89-2	23
			Methyltris(trimethylsiloxy)silane	130466	017928-28-8	17
22	37.449	1.24	C:\Database\NIST05.L			
			Silicic acid, diethyl bis(trimethylsilyl) ester	121708	003555-45-1	40
			1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	153472	101869-40-3	33
			3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyl trisiloxane	138615	018082-56-9	32

Figure B-10. Incense (INC-Fa, Mainichi-koh) PM2.5 F2, PMeq injected = 2.4 µg



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.723	53.67	C:\Database\NIST05.L			
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	83
			2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-1,3-bis(trimethylsilyl)-	155144	052988-92-8	38
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	35
2	17.738	7.69	C:\Database\NIST05.L			
			Naphthalene, 2-methoxy-	28427	000093-04-9	91
			Naphthalene, 2-methoxy-	28426	000093-04-9	90
			Naphthalene, 2-methoxy-	28424	000093-04-9	83
3	17.953	28.64	C:\Database\NIST05.L			
			2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	140587	1000275-63-1	47
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	187800	071579-69-6	38
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	38
4	19.938	10	C:\Database\NIST05.L			
			5-Hydroxy-1-(3-isopropoxy-propyl)-2-methyl-1H-benzo[g]indole-3-carboxylic acid methyl ester	154964	1000297-43-9	5
			Naphthalene, 2-(4-cyanophenyl)-6-nonyl-	155028	100808-10-4	5
			Glaucine	154943	000475-81-0	5

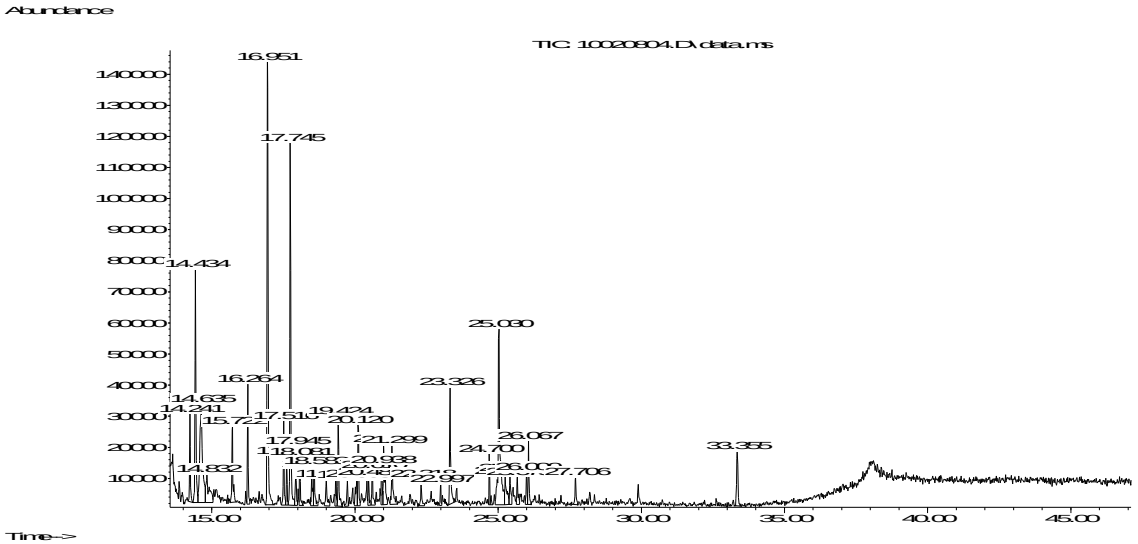
Figure B-11. Incense (INC-Fa, Mainichi-koh) PM2.5 F3, PMeq injected = 2.3 µg



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.722	10.9	C:\Database\NIST05.L			
			Cyclohexasiloxane, dodecamethyl-	179151	000540-97-6	90
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	87
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	86
2	17.953	5.26	C:\Database\NIST05.L			
			2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	140587	1000275-63-1	37
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetra siloxane	187800	071579-69-6	32
			trans-4-(2-(5-Nitro-2-furyl)vinyl) -2-quinolinamine	112453	000847-10-9	27
3	19.922	1.98	C:\Database\NIST05.L			
			8-Furan-2-yl-3,3-dimethyl-6-morpho lin-4-yl-3,4-dihydro-1H-thiopyrano [3,4-c]pyridine-5-carbonitrile	154877	1000274-37-6	38
			Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	161132	003618-20-0	32
			N-(Trifluoroacetyl)-N,O,O',O''-tetrakis (trimethylsilyl)norepinephrin	187076	1000072-26-7	32
4	21.168	7.69	C:\Database\NIST05.L			
			Octanal, 2-(phenylmethylene)-	68637	000101-86-0	94
			1,2,4-Metheno-1H-cyclobuta[b]cyclo penta[d]furan, 2,2a,3a,4,6a,6b-hexahydro-3a-methyl-	29519	078323-74-7	32
			Benzene, (1-chloro-2,2-dimethylcyclo propyl)-	42873	013153-97-4	27

5	21.445	71.25	C:\Database\NIST05.L			
			Benzyl Benzoate	65863	000120-51-4	95
			Benzyl Benzoate	65862	000120-51-4	94
			Benzyl Benzoate	65861	000120-51-4	86
6	22.322	2.92	C:\Database\NIST05.L			
			1-Tripropylsilyloxyoctane	116055	1000279-12-0	25
			2-Propenamide, 2-cyano-N,N-dimethyl-3-[4-(dimethylamino)phenyl]-	87316	125535-35-5	25
			3,10-Dimethyl-pyrido[3,2-g]pteridin-2,4(3H,10H)-dione	87153	1000286-78-9	16

Figure B-12. Incense (INC-Fa, Mainichi-koh) PM2.5 F4, PMeq injected = 2.0 µg



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	14.246	2.11	C:\Database\NIST05.L			
			Cyclotetrasiloxane, octamethyl-	122479	000556-67-2	86
			1-Thia-2-azacyclopenta[a]anthracene -3,6,11-trione	112516	1000303-19-5	59
			5H-Naphtho[2,3-c]carbazole, 5-methyl-	112706	100025-44-3	53
2	14.43	6.38	C:\Database\NIST05.L			
			Benzene, 1-phenyl-4-(2-cyano-2-phenylethenyl)	112707	027869-56-3	59
			7H-Dibenzo[b,g]carbazole, 7-methyl	112705	003557-49-1	53
			6-Chloro-3-ethyl-2-methyl-4-phenyl quinoline	112624	022609-09-2	45
3	14.63	7.83	C:\Database\NIST05.L			
			Cyclotetrasiloxane, octamethyl-	122479	000556-67-2	47
			Benzoic acid, 4-methyl-2-trimethyl silyloxy-, trimethylsilyl ester	121893	1000153-59-3	47
			Morphinan, 7,8-didehydro-3-methoxy -17-methyl-6-methylene-, (-)-	112687	001816-06-4	38
4	14.83	2.61	C:\Database\NIST05.L			
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	177117	000995-82-4	38
			3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	147384	018030-67-6	37
			Silanamine, N-[2,6-dimethyl-4-[(tri methylsilyl)oxy]phenyl]-1,1,1-trimethyl	112442	072088-09-6	35

5	15.722	2.55	C:\Database\NIST05.L			
			Cyclohexasiloxane, dodecamethyl-	179151	000540-97-6	90
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	78
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	52
6	16.261	2.92	C:\Database\NIST05.L			
			Phenol, 2,6-dimethoxy-	26275	000091-10-1	81
			Phenol, 2,6-dimethoxy-	26272	000091-10-1	81
			3-Amino-2,6-dimethoxypyridine	26163	028020-37-3	62
7	16.953	13.72	C:\Database\NIST05.L			
			Vanillin	24745	000121-33-5	97
			Vanillin	24742	000121-33-5	96
			Propenylguaethol	24750	000094-86-0	96
8	17.522	2.19	C:\Database\NIST05.L			
			Dehydroacetic Acid	35405	000520-45-6	58
			Phenol, 4-methoxy-3-(methoxymethyl)-	35516	059907-65-2	53
			2,5-Dimethoxybenzyl alcohol	35491	033524-31-1	52
9	17.615	1.12	C:\Database\NIST05.L			
			Eugenol	31716	000097-53-0	64
			3-Allyl-6-methoxyphenol	31764	000501-19-9	62
			Phenol, 2-methoxy-5-(1-propenyl)-,(E)-	31884	019784-98-6	62
10	17.738	10.32	C:\Database\NIST05.L			
			Propanal, 2-methyl-, oxime	1863	000151-00-8	38
			Bromoacetic acid, pentyl ester	63173	052034-03-4	37
			1,2,4-Cyclopentanetrione, 3,3-dimethyl-	17737	017530-56-2	37
11	17.938	1.46	C:\Database\NIST05.L			
			Pentasiloxane, dodecamethyl-	166194	000141-63-9	43
			2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	140587	1000275-63-1	38
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	177117	000995-82-4	38
12	18.076	1.37	C:\Database\NIST05.L			
			Ethanone, 1-(4-hydroxy-3-methoxy phenyl)-	34048	000498-02-2	72
			Ethanone, 1-(3-hydroxy-4-methoxy phenyl)-	34041	006100-74-9	72
			Ethanone, 1-(4-hydroxy-3-methoxy phenyl)-	34047	000498-02-2	72
13	18.491	1.18	C:\Database\NIST05.L			
			5-tert-Butylpyrogallol	44207	020481-17-8	64
			Ethanone, 1-(2,6-dihydroxy-4-methoxy phenyl)-	45095	007507-89-3	59

			3-Isopropyl-1-methyl-4-methylamino -pyrrole-2,5-dione	45147	1000296-12-2	58
14	18.584	1.45	C:\Database\NIST05.L			
			2-Methylthianaphthene-1,1 dioxide	43663	006224-55-1	59
			Homovanillyl alcohol	35472	002380-78-1	53
			Ethyl homovanillate	63950	060563-13-5	50
15	18.999	1.11	C:\Database\NIST05.L			
			2,3,5,6-Tetrafluoroanisole	43464	002324-98-3	53
			2,3,5,6-Tetrafluoroanisole	43465	002324-98-3	53
			2,4(1H,3H)-Pyrimidinedione, 5-(tri fluoromethyl)-	43291	000054-20-6	43
16	19.337	0.88	C:\Database\NIST05.L			
			Phthalic acid, allyl ethyl ester	80915	033672-94-5	64
			Diethyl Phthalate	72412	000084-66-2	64
			Phthalic acid, 2-methoxyethyl nonyl ester	152636	1000315-80-5	53
17	19.43	2.43	C:\Database\NIST05.L			
			Benzene, 1-fluoro-3-(phenylmethyl)	47483	001496-00-0	53
			Benzene, 1-fluoro-3-(phenylmethyl)	47482	001496-00-0	53
			4-Fluorodiphenylmethane	47480	000587-79-1	53
18	19.737	0.73	C:\Database\NIST05.L			
			2,6a-Methano-6aH-indeno[4,5-b] oxirene, octahydro-, (1a.alpha.,2.beta., 3a.alpha.,6a.beta.,6b.alpha.)-	22948	016489-32-0	27
			Naphthalene, 2-(1,1-dimethylethyl) decahydro-4a-methyl-	63063	054934-96-2	22
			Tricyclo[4.4.0.0(2,8)]dec-3-en-5-o	22812	1000193-38-7	18
19	20.06	1.11	C:\Database\NIST05.L			
			Quinoline, 2,4-dimethyl-	27984	001198-37-4	38
			Quinoline, 4,8-dimethyl-	27978	013362-80-6	38
			2,8-Dimethylquinoline	27973	001463-17-8	38
20	20.122	2.44	C:\Database\NIST05.L			
			Benzaldehyde, 4-hydroxy-3,5- dimethoxy-	45075	000134-96-3	93
			Benzaldehyde, 4-hydroxy-3,5- dimethoxy-	45077	000134-96-3	91
			Benzaldehyde, 4-hydroxy-3,5- dimethoxy-	45076	000134-96-3	90
21	20.476	0.77	C:\Database\NIST05.L			
			Isolongifolan-8-ol	72917	001139-08-8	41
			Patchouli alcohol	72916	005986-55-0	35
			Patchouli alcohol	72914	005986-55-0	30

22	20.614	1.19	C:\Database\NIST05.L			
			Phenol, 2,6-dimethoxy-4-(2-propenyl)-	52459	006627-88-9	64
			Phenol, 2,6-dimethoxy-4-(2-propenyl)-	52464	006627-88-9	53
			Benzofurazan, 4-(methylamino)-7-nitro	53150	018378-29-5	50
23	20.937	1.25	C:\Database\NIST05.L			
			Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	53990	002478-38-8	50
			Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	53989	002478-38-8	40
			Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-2,5-dimethyl-, hydroxide, inner salt	44143	023933-08-6	17
24	21.014	2.61	C:\Database\NIST05.L			
			4-Hydroxy-2-methoxycinnamaldehyde	41242	127321-19-1	52
			(3-Methyl-1-benzothiophen-2-yl) methanol	41312	003133-88-8	43
			Benzene, 1,2-dimethoxy-4-(2-propenyl)-	41487	000093-15-2	38
25	21.306	2.31	C:\Database\NIST05.L			
			Thiophene, 2-isobutyl-5-isopentyl-	64298	004806-10-4	59
			2-Pentanone, 1-(2,4,6-trihydroxyphenyl)	63972	1000116-22-3	58
			1-(1-Hydroxybutyl)-2,5-dimethoxybenzene	64113	149083-03-4	58
26	22.322	0.93	C:\Database\NIST05.L			
			Ethanone, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-	97613	001506-02-1	53
			7-Acetyl-6-ethyl-1,1,4,4-tetramethyl tetralin	97609	000088-29-9	52
			7-Acetyl-6-ethyl-1,1,4,4-tetramethyl tetralin	97608	000088-29-9	47
27	22.999	0.56	C:\Database\NIST05.L			
			Hexadecanoic acid, methyl ester	105639	000112-39-0	72
			Pentadecanoic acid, 14-methyl-, methyl ester	105661	005129-60-2	59
			Hexadecanoic acid, methyl ester	105644	000112-39-0	59
28	23.322	3.94	C:\Database\NIST05.L			
			1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	110588	000084-69-5	35
			2(3H)-Benzofuranone, 3,3-dimethyl-5-[(methylsulfonyl)oxy]-	95637	026244-33-7	35
			Phthalic acid, isobutyl non-5-yn-3-yl ester	149820	1000315-18-8	35
29	24.706	1.2	C:\Database\NIST05.L			
			1,1'-Bicyclohexyl, 4-methyl-4'-propyl-	73074	092343-70-9	43
			Oleic Acid	113353	000112-80-1	38
			1-Tetradecanol	67335	000112-72-1	30

30	25.029	9.63	C:\Database\NIST05.L			
			6-Octadecenoic acid, (Z)-	113359	000593-39-5	98
			Oleic Acid	113353	000112-80-1	94
			9-Octadecenoic acid, (E)-	113363	000112-79-8	93

Appendix C. Recommended Summary Procedures

The following is a recommended summary procedure, based on the findings of this project:

1. Collect indoor source PM on filters using size selective devices. PM2.5 is appropriate for candle and incense PM while PM10 is appropriate for cooking and woodsmoke PM. Collect these samples near the source.

2. Extract filters with organic solvent (typically methanol followed by dichloromethane) using shaking followed by sonication. The extract is then recovered by removing the solvent under a stream of nitrogen and re-dissolving in dimethyl sulfoxide (DMSO). A workable final concentration of the PM in the culture system is 10 µg/ml equivalent of PM.

3. Treat human macrophage cells (U937) and human lung cells (NCI H441) with PM extracts for 24 hr. Isolate mRNA from the cells.

4. Determine molecular expression of markers for inflammation cytochrome P4501A1 (CYP1A1), cyclooxygenase 2 (COX-2), and interleukin 8 (IL-8) in the macrophage cells. Determine molecular expression of markers for inflammation CYP1A1, COX-2, and mucin-5AC (MUC5AC) in the human lung cell line.

5. Compare the effects of indoor PM and positive controls on marker gene expression. Recommended positive controls include: 2,3,7,8-Tetrachlorodibenzodioxin (TCDD), Ambient Air PM (SRM 1650a) and Diesel PM (SRM 2975).

6. Chemically analyze PM extracts using gas chromatography/mass spectrometry (GC/MS) for polycyclic aromatic hydrocarbons (PAHs). Add internal standards and quantitatively determine compounds present using Selective Ion Monitoring (SIM). For general chemical characterization, extracts can be analyzed directly using Total Ion Chromatographic (TIC) scans.

7. Chemically extract vapor phase compounds which were collected in series with the PM samples. Chemically analyze these extracts using GC/MS for PAHs using internal standards and SIM analyses.

8. Chemically characterize qualitatively the PM and vapor phase extracts using GC/MS and Total Ion Chromatography.